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Biomass and mass balance isotope content of mussel seep populations

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BIOMASS AND MASS BALANCE ISOTOPE CONTENT OF MUSSEL SEEP POPULATIONS

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Abstract

Cold seep mussels, *Bathymodiolus childressi*, are common cold seep constituents that form large populations at upper continental slope (500-1000 m) cold seep sites in the Northern Gulf of Mexico. These mussels utilize methane present through symbiotic relationships with methanotrophic bacteria. This study uses a coupled isotope technique to determine the relative incorporation of respiratory carbon in the shell as a measure of the availability of methane between different seep sites. This method indicates a higher abundance of methane at the Brine Pool site than at the Bush Hill site which appears significantly more resource limited and that changes in methane availability can arise on both decadal and yearly time scales. The method has implications for determining long term methane abundances in both archived samples and disarticulated shells with a relative minimum of additional cost. Additionally, analysis of the means and standard deviations of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ of mussel soft tissue can provide indications of the presence and variability of those resources across time and space. These analyses indicate the utilization of unique resources, specifically ammonium and thermogenic or biogenic methane, between the two sites. The difference in resources at each site can support further development of unique mixing models for each site that utilize the resources present and not a single blanket analysis using similar resource values for all cold seep sites.

1 Chapter 1: Introduction to the Hydrocarbon Seep System of the Northern Gulf of Mexico

1.1 In the Context of Deep Trophic Systems

On the deep seafloor from continental margins to abyssal settings (100-5000 m depth), most heterotrophic organisms rely on the particulate organic carbon (POC) flux from near-surface photosynthetic productivity for their energetic needs. Typically, the labile carbon content of this flux decreases exponentially with depth with a parallel decline in benthic metazoan biomass. This has been confirmed to be a global pattern (Rex et al. 2006) which is also found in the Gulf of Mexico (Wei et al. 2010). In contrast to this POC trophic system are chemosynthetic communities such as hydrothermal vents and cold seeps which derive a large portion of their energetic needs from the reliance on chemosynthetic free-living and symbiotic microbes oxidizing sulfide and/or methane present in these extreme environments (Petersen and Dubilier 2009).

The cold seeps in the Northern Gulf of Mexico have been particularly well studied due to the interest in oil and gas production in this region. Investigations to date have found large variations in seep-community size, species composition, and isotopic compositions in species assemblages (Cordes et al. 2009). As with the heterotrophic fauna, seep assemblages undergo a distinct bathymetric zonation when the full depth range is examined, but on a more local scale temporal and spatial variation must be related to variable local seepage rates (Cordes et al 2010b, 2010c). Based on a site-to-site comparison of features, a sequence of seepage stages has been proposed which envisions seeps as initiating with a fluid-dominated phase and terminating with a mineral-prone phase (Roberts and Carney 1997). Transitions between phases have yet to be observed at individual seep sites, but it can be assumed that ecological conditions also change. Thus site-to-site and within-site variations may reflect differences in seep maturation.

The predominant symbiont-bearing species responsible for the large increases in biomass found at both hydrothermal vent and cold seep sites include bathymodiolin mussels, vesicomyid clams, and vestimentiferan tubeworms. Species assemblages associated with cold seeps have typically been divided into two categories, those endemic to the seep area and vagrants that are loosely associate with the area, but are not reliant upon chemosynthetic production to a large degree (Bergquist et al. 2003; R. S. Carney 1994). Endemic foundation species consist of tubeworms that house thiotrophic symbionts and mytilid mussels with both solely methanotrophic symbionts and a mix of methanotrophic and thiotrophic symbionts.

1.2 Consequences to Stable Isotope Composition

The decoupling of the reliance on POC flux allows these communities to form large amounts of biomass containing thousands of individuals spread out in a very patchy nature from tens to hundreds of kilometers apart, in the case of cold seeps, to thousands of kilometers apart in the case of hydrothermal systems. POC is a smaller source of nutrition in these systems when

compared to the background deep water systems (Levin and Dayton 2009). Increased biomass should lead to the support of increased endemic trophic levels and to the support of a wide range of transient consumers, leading to the theory of both ecosystem types as oases of chemosynthetic production. Chemosynthetic production may or may not be incorporated into the surrounding area through both transient heterotrophs and endemic consumers, free living bacteria, and hydrocarbon associated plumes (Bergquist et al. 2007; R. S. Carney 1994; R. S. Carney 2010; Macavoy et al. 2003). The latter two studies have found some reliance on cold seep production by detritivores closely associated with seep fauna, but little evidence exist showing widespread chemosynthetic production incorporated in a significant way to the surrounding heterotrophs.

Carbonates precipitate at seeps as a product of the microbial oxidation of hydrocarbons (H. Roberts et al. 1989). This causes the sites to become suitably hard for there to be a transition of fauna. The transition begins with the colonization by mussels taking advantage of the hard substrate and available methane. As sulfide becomes more abundant in the system, tubeworms begin to colonize and use the available hydrogen sulfide in the underlying sediments (Bergquist et al. 2003). The communities that are associated with mussel beds show a diversity maximum at 1400 m and a minimum at 600 m, consisting of mainly *Alvinocaris stactophila* (endemic shrimp) and *Bathynerita naticoidea* (endemic grazing snail) (Bergquist et al. 2005; Cordes et al. 2010b). Tubeworm communities show a series of successional stages with increasing age as biomass decreases and sulfide is exhausted in the upper sediments below the organism (Bergquist et al. 2003).

1.3 Underlying Geological Processes as a Source of Variation.

The critical biogeochemical process causing the transitions within the seep sequence is microbial anaerobic oxidation of hydrocarbons and the deposition of authigenic carbonates. Deposition of these carbonates is a common feature of methane-charged marine sediments and is widely studied (Gieskes et al. 2005; Mansour and Sassen 2011; Naehr et al. 2007). From an ecological perspective the process can be seen as causing a change from soft to hard bottom and altering the rates and composition of seeping materials (Chen et al. 2004; Cordes et al. 2010a; Neurauter and Roberts 1994). The seeping fluids can be heterogeneous mixtures of dissolved or gas-phase methane, liquid hydrocarbons, hydrogen sulfide and in some geological settings brine (Bidigare et al. 1987).

The geological setting in the Northern Gulf of Mexico is one of extreme complexity, due to the formation of salt sheets (Louann/Werner formations) during the Late Triassic which were subsequently buried under siliciclastic sediments (Galloway et al. 2000). This loading of sediment on top of salt has caused much deformation and upward thrusting of salt diapirs which have been accompanied with upward migration and trapping of hydrocarbons as they encounter the impermeable salt (Sassen et al. 2004). Fractures in the overlying sediments associated with the deformation by salt diapirs make efficient conduits to the seafloor for underlying deep hydrocarbons and ultimately result in the expression of venting hydrocarbons in the form of oil, gas, and brine mixtures.

Brines 3 to 5 times the density of seawater may have multiple origins and be either fossil water or seawater in contact with dissolving evaporites. These brines are predominately anoxic, and show a diverse chemical makeup depending on the minerals that interacted during the siliciclastic sediment compaction surrounding the salt body and the bacterial activity that occurred during both formation and migration to the surface. At Orca Basin in the Gulf of Mexico, the brine is anoxic due to the existence of iron oxides scavenging oxygen out of the brine (Aharon et al. 1992). This is not the case at GC 233 (Brine Pool NR-1) where there is little expression of iron, but a large concentration of ammonium at 10863 uM (Joye et al. 2005) that has either arisen from the surrounding sediments or is a result of decaying animal carcasses trapped within the brine.

Hydrocarbons presenting at the seafloor are evident as oily sediment, gas bubbling, and formations of gas hydrate. Gas hydrate is an ice-like crystalline structure with multiple lattice configurations that trap both hydrocarbon and non-hydrocarbon gasses. Accumulations of gas hydrate within the sediments surrounding seeping hydrocarbons is common once the depth exceeds 450-500 m and it is not uncommon for outcroppings and mounds of gas hydrate to form (Sassen et al. 1999). Gas hydrate features are commonly observed with bacterial mats mainly composed of sulfur oxidizing bacteria (sulfur oxidizing bacteria) such as *Beggiatoa* or *Thioplica* capping them.

Bacterial assemblages use reduced hydrogen sulfide formed by the microbial community in the shallow sediments beneath them as their main energy source and are using nitrate as their electron acceptor (Dale et al. 2009; Lloyd et al. 2010), leading to a novel source for depleted $\delta^{34}S$ in chemosynthetic environments. These hydrates are made up of methane from two sources, biogenic and thermogenic methane, the differences between them are described in more detail in the following section on stable isotopes (Aharon 1994). Continental margin settings are extremely heterogeneous with regards to available chemical species, fluid flows, and biological constituents that can change on a meter to a regional scale basis allowing for the existence of multiple reduction pathways in a manner not seen in other habitats (Cordes et al. 2010c). This is often due to the extremely complex geologic structure of passive margins. Usually large amounts of organic material are being reworked in the sediments as migration of salt compresses and heats them, giving rise to a reducing environment and allowing for the creation of various sulfide compounds and an abundance of methane.

1.4 Upper-slope Seep Foundation Species *Bathymodiolus childressi*

1.4.1 General Biology

As of 2010, 51 nominal living and fossil species had been assigned to the genus *Bathymodiolus* (Saeter et al. 2010), which is comprised of species of mussels that have a widespread presence at both hydrothermal vents and cold seep sites, where they account for a large percentage of the biomass and act as a foundation species providing structure.

Upper-slope seep communities in the Northern Gulf of Mexico often contain beds of the mytilid mussel *Bathymodiolus childressi* that rely on chemosynthetic symbionts housed in stacked internal membranes in the gills to oxidize methane and provide energy to its host. As yet reported only from the Northern Gulf of Mexico, it is still considered to be an endemic seep constituent (R. S. Carney 1994; Childress et al. 1986). The local populations at widely scattered seep sites (527-2222 m) show no differentiation among sites indicating a single interbreeding species population (S. L. Carney et al. 2006). This is consistent with net sampling and laboratory rearing studies which show considerable larva dispersion capacity (Arellano and Young 2009).

Two important aspects of feeding of larval and juvenile feeding remain unanswered. The first is whether larvae and juveniles participate in the POC trophic system, and the second is when does the symbiotic relationship begin? Feeding was never observed during larval culture studies, although egg, larval and early shell size all pointed to planktotrophic existence (Arellano and Young 2009). Microscopic and stable isotope analyses of post-larval and juvenile species of the related *B. azoricus* and *B. heckeri* found well-developed populations of symbionts and no evidence of residual POC-based tissues (Salerno et al. 2005). It remains uncertain whether the symbionts are transferred from parent to larvae or whether the bacteria are acquired after settlement from the surrounding environment (Duperron 2010). For the size range of mussels included in the present study, carryover of larval POC tissue is not considered a source of variation.

Adult utilization of POC ingested by filter feeding is considered to be a likely source of tissue stable isotope variation. While *Bathymodiolus childressi* does harbor symbiotic microbes primarily in gill tissue, the species possess a fairly typical modiolin feeding architecture and functional gut. Kept in culture, specimens do filter and assimilate suspended bacteria and algae (Page et al. 1990). Shipboard feeding studies using mussels from Green Canyon 233 (Brine Pool) and *in situ* collected seawater showed retention of the ambient suspended microbes with bacteria making the largest potential contribution of carbon and nitrogen. Assimilation was estimated rather than being measured and assimilation of bacteria is considered to be an important source of nitrogen supplementing that obtained from symbionts (Pile and Young 1999). Seeping methane has been shown to be very efficiently assimilated via the symbiotic route with approximately 51% being retained in mussel tissue from specimens from Green canyon 184/5 (Kochevar et al. 1992). An additional suggestion that filter feeding may be important is a possible link between the periodicity of mussel spawning and the seasonal pulses of phytoplankton productivity that align with the spawning of *B. childressi* (Tyler et al. 2007).

1.4.2 Symbiotes

Species of the genus *Bathymodiolus* host various combinations of methanotrophic and thiotrophic symbionts, but *Bathymodiolus childressi* has only been demonstrated to host a Type 1 methanotroph *Gammaproteobacteria* (Domain *Archea*) (Duperron et al. 2007; Kellermann et al. 2012). These symbionts utilize a specific enzyme, *methane monooxygenase,* to facilitate the introduction of an oxygen atom into the methane molecule. This makes them obligate aerobes

and obligate C_1 utilizers without the ability to break C-C bonds. Methanotrophs also have the ability to oxidize ammonia via *methane monooxygenase* to nitrate, although these two substrates (CH_4 and NH_4^+) have a competitive interaction, meaning that high levels of ammonia can be toxic to these symbionts (Madigan and Martinko 2006). The symbionts use methane as both an electron donor and a carbon source with oxygen as the electron acceptor. This means that these bacteria must be in a habitat with access to both free methane and oxygen simultaneously. The mussel provides a stable environment and access to a steady flow of methane and oxygen for the symbionts in the bacteriocytes in their gills as they position themselves favorably. Reliance on the processing of methane leads to the ranges for $\delta^{13}C$ in mussel tissue being closely associated with site-specific methane sources. Populations that are reliant on biogenic methane are more depleted (-50 to -70‰) than those reliant upon thermogenic methane (-20 to-50‰) (Cavanaugh et al. 1987; Kennicutt et al. 1992). The extent of filter feeding is not clear due to the ability of both the mussel and the symbiont to take up nitrate and ammonia directly from the surrounding environment (Lee and Childress 1996), confounding simple measurement of nitrogen contribution from particulate filtering (Page et al. 1990).

1.4.3 Condition Variation Between Sites

Consistent with the geological expectation that seepage composition and rate changes among sites and through time, mussel populations can be expected to be in different condition among sites and through time. This has been demonstrated between sites employing traditional condition indices for mollusks such as water and glycogen content and the ratio of ash-free dry tissue to shell volume. (Nix et al. 1995; Smith et al. 2000). In general, condition indices indicated more favorable conditions at the Brine Pool (GC 233) than at Bush Hill (GC 185). These methods fail to adequately address the causation of the differences between the condition of the mussels, leading to speculation that resource availability (CH_4, O_2) or exposure to hydrogen sulfide was the ultimate cause.

1.4.4 Shell

Shell growth for *Bathymodiolus childressi* (then called Seep Mytilid Ia) has been characterized using mark/recapture experiments at three non-brine seep sites and the Brine Pool (Nix et al. 1995; Smith et al. 2000). Bush Hill growth results were complex with some of 47 animals recaptured after one year intervals losing 1 or 2 mm in length. The maximum gain was ~5 mm for a 50 mm specimen. The other two non-seep sites showed a more typical decrease in shell growth with increasing shell length and up to 18 mm of growth for a 45 mm animal. Age estimates of the largest specimens were estimated to be in excess of 65 years based on parameters derived from Ford-Walford plots. A similar age assessment was not carried out for the Brine Pool. Growth rates were inversely size related with zero annual growth occurring at approximately 120 mm length. Maximum additions of ~18 mm were observed in smaller animals. These basic results were repeated subsequently at the Brine Pool and Bush Hill (Dattagupta et al. 2004).

A strong dependence of shell deposition on the availability of methane has been shown in a single study of aquarium-maintained specimens. Without additional food sources, shell growth strongly linked to methane intake became essentially zero when methane was discontinued (Cary et al. 1988).

1.5 State of Knowledge of the Two Sites Sampled in This Study

1.5.1 Brine Pool (Green Canyon 233)

This site is a brine pool at a depth of 650 m with a surface area of 190 m^2 that is located 230 kilometers southwest of the southwest pass of the Mississippi River (27°43'24" N, 91°16'30" W) (Fig. 1.1). The pool has a salinity of 130‰ that is a result of the exposure of the salt formation below the seafloor to seawater (Dattagupta et al. 2007; Joye et al. 2005; I. Macdonald et al. 1990). The brine pool collects within a pockmark set into a topographic high that is higher on the North end, while the south end slopes gradually, allowing for the overflow of brine downslope. There is a central vent towards the North end of the pool from which methane saturated fluid flows upward and releases bubbles of methane. The density difference between the seawater and the brine is high enough to keep the brine mostly confined to the central depression with diffusion occurring at the interface between the brine and overlying seawater. The density structure has been previously indicated to be stable across an eight year time period (Joye et al. 2005). As water was sampled in zones more towards the edges of the pool, elevated levels of hydrogen sulfide were present, but never below a salinity of 39 ‰ (Smith et al. 2000). The composition of the methane in the gas bubbles was predominately CH_4 (76.3%) with a concentration ranging from 33-44 M/m^{-3} with a $\delta^{13}C$ value of -63.8‰ in water samples taken across the pool (I. R. Macdonald et al. 1990; Smith et al. 2000). The brine pool is surrounded by a ring of mussel bed that ranges from 3 to 7 m in width and has a surface area of ~540 m^2 (Smith et al. 2000).

1.5.2 Bush Hill (Green Canyon 185)

The Bush Hill site is a liquid petroleum-dominated topographic high in 540-580 m of water along a fault adjacent to a salt diapir (Macdonald et al. 2003). Faunally, it is dominated by dense assemblages of tube worms with mussel beds scattered among them. Streams of methane bubbles and oil stained sediment have been observed throughout the site and there are numerous carbonate outcrops. This site is low in sulfide and has a relatively low concentrations of methane (0- < 60 mmol m^{-3}) with yearly sampling (1992 - 1993; (Nix et al. 1995) 1997 - 1998; (Bergquist et al. 2004b)) and is hypoxic (0.057- 0.226 M m^{-3} oxygen (Bergquist et al. 2004b)). The mussels at this site are predominately large (> 5.5 cm) and have a condition poor index (CI) (Nix et al. 1995).

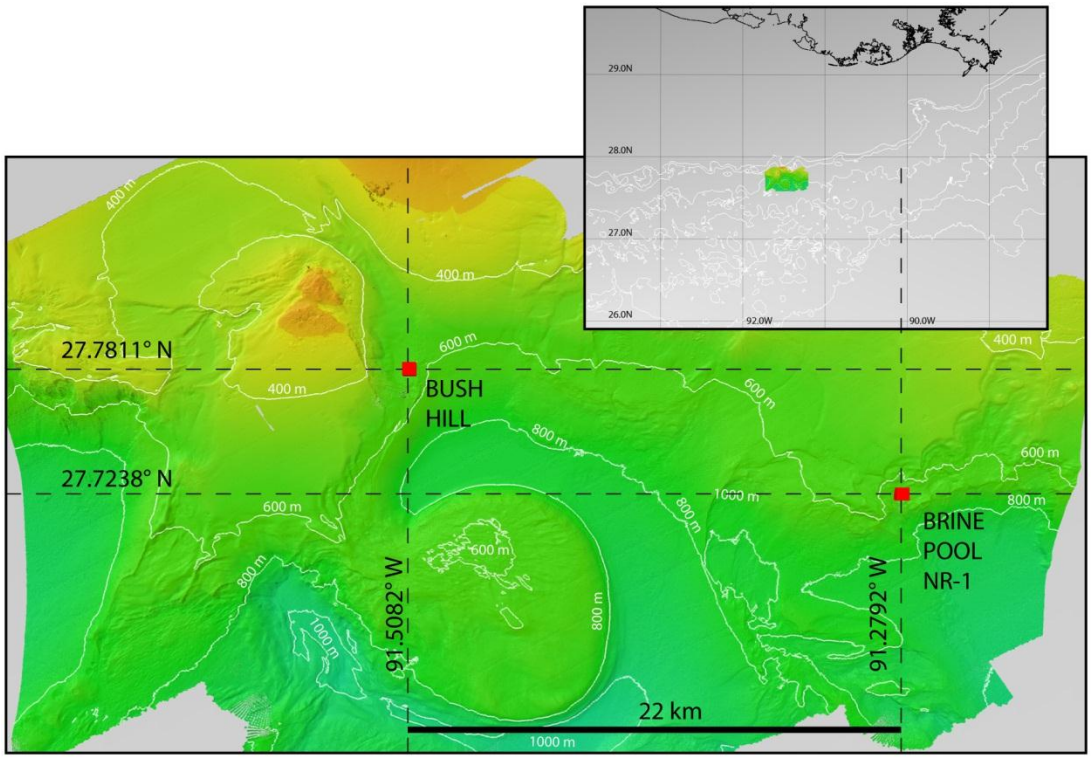

Figure 1.1: Site Locations for the Brine Pool (NR-1) and Bush Hill. These sites are located approximately 230 km from the southwest pass of the Mississippi River (inset) and bathymetric contours are in meters. Digital terrain model based on multibeam data provided by the NOAA Okeanos Explorer program to R. S. Carney.

1.6 Sampling Design

Mussels sampled in this study were originally collected as part of the Chemo - 1 project on cold seep sites 1989-1992 and were intended as general assemblage surveys. The original sampling design sought to detect between and within mussel bed variations. Therefore mussel beds at different sites were sampled at both the outer edges and at the interior. The mussel *Bathymodiolus childressi* and snail *Bathynerita naticoidea* dominated the samples. The interior zone is the area in the ring of mussels closest to the pool of brine in the center at the Brine Pool site, while the outer zone (Edge) was the furthest from the Brine. The interior zone at the Bush Hill site were taken in the innermost areas of the mussel beds present there, while the outer zone (Edge) were taken at the edge of the mussel beds.

1.7 Stable Isotopes

Isotopes exist for most elements and come about due to the presence of extra neutrons in the nucleus of an atom with the same number of electrons. This leads to the existence of relatively lighter and heavier isotope forms (^{13}C vs. ^{12}C vs. ^{14}C) that proceed through biological and chemical processes at different rates due to the increased mass of the extra neutron(s). Stable isotopes are those forms that exist in the natural environment and are not radioactive in nature. Stable isotopes are commonly referred to as HCNOS isotopes in biological studies and

these are the isotopic forms of hydrogen, carbon, nitrogen, oxygen, and sulfur that are commonly used to identify biological processes in natural systems (Brian Fry 2006). HCNOS elements are of particular interest because they are necessary for all forms of life on the planet and as such are particularly useful tools in tracking the flow of essential elements through a multitude of ecological and physical processes. Stable isotopes of carbon and nitrogen have been utilized in many ways: to delineate food webs and decipher who's eating who leading to the 'you are what you eat' principle (Peterson and Fry 1987), identified the relative transience of fish in estuaries (B. Fry and Chumchal 2011), and have had implications for global warming through the use of biogenic carbonates as a proxy record for global warming and cooling trends (Arbuszewski et al. 2010; Pucéat et al. 2007). There are many current applications for these tools, since they allow for the relatively cheap tracing of elements through both biological and physical processes without the need for expensive or radioactive tracers. This thesis will address the usage of naturally occurring carbon, nitrogen, and sulfur within the Northern Gulf of Mexico populations of the mytilid bivalves *Bathymodiolus childressi*.

Stable isotope measurements are reported in δ (del) notation, that is to say, that they are reported in standard notation as:

$$\delta = \{[(^{H}F/^{L}F)\text{sample}/(^{H}F/^{L}F)\text{standard}]-1\} * 1000$$

with $(^{H}F/^{L}F)$ being the fractional abundance of heavy and light isotope of both the sample and the standard. This formula is a ratio of ratios and yields a very small number that becomes manageable with the multiplication of 1000. The δ notation is then in the per mil (‰) basis, that has fewer decimal digits and thus is easier to evaluate, which allows for the amplification of very small differences into a more familiar and manageable scale. The standards that samples are measured against are as follows, for carbon, PeeDee Belemnite (PDB), for nitrogen, air, and for sulfur, Canyon Diablo Troilite (CDT) (Brian Fry 2006). These standards have been meticulously established in order to provide lab to lab continuity and quality assurance for stable isotope analysis and reference materials are currently maintained by the International Atomic Energy Agency.

1.7.1 Carbon

1.7.1.1 General

On the ecosystem scale, carbon is utilized to trace the relative contribution of different dietary sources to the diet of food web constituents. This is usually accomplished through the use of a two source mixing model:

$$\delta^{13}C_{organism} = (f * \delta^{13}C_{source1}) + ((1-f) * \delta^{13}C_{source2})$$

where $f * \delta^{13}C_{source1}$ is the relative contribution of one end member and the remainder of the diet (1-f) is composed of a second end member, $\delta^{13}C_{source2}$. The $\delta^{13}C_{organism}$ is the result of the mixture of both food sources and as such falls in between the $\delta^{13}C$ values of the two end

member sources. This simple model is rarely an accurate case in nature, where multiple dietary sources and extremely complex food webs are the standard in most ecosystems. This method can generate more questions than answers, especially if there are unaccounted unique sources that are distinct in their isotope values in the system (Brian Fry 2006).

1.7.1.2 Methane

In cold seeps, methane is a source of extremely depleted $\delta^{13}C$ and it can range from -110‰ to -50‰ for bacterially derived sources and -50‰ to -20‰ for thermogenic sources (Whiticar 1999). The primary difference between these varieties of methane is the way that they are created. Thermogenic methane is the result of the heating of organic material, typically deep underground under elevated temperature and pressure, which is heated to the point where the long chain carbon molecules begin to break apart and light hydrocarbons form. This results in a kinetic isotope effect (KIE) that leaves the resulting hydrocarbons lighter than the source material as ^{12}C proceeds through these processes fractionally quicker than does ^{13}C. Bacterially derived sources of methane are typically far more depleted in $\delta^{13}C$ than thermogenic methane as a result of the larger fractionations associated with both types of biogenic methanogenesis (methyl-fermentation to CO_2 and carbonate reduction to CO_2) (Alperin and Hoehler 2009; Templeton et al. 2006; Whiticar 1999). Differentiating between the two pathways for methanogenesis requires the use of both $\delta^{13}C_{CH4}$ and δD_{CH4} and is important due to the different fractionations that occur as a result of distinctly different biological pathways for methane oxidation. Bacterially mediated methanogenesis can arise from two different pathways.

Carbonate reduction proceeds via the pathway:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

which produces a more depleted $\delta^{13}C_{CH4}$ (-60‰ to -110‰) with less depleted δD_{CH4} (-150‰ to -250‰) and acetate fermentation:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

which produces a relatively less depleted $\delta^{13}C_{CH4}$ (-50‰ to -70‰) and a more depleted δD_{CH4} (-300‰ to 400‰).

1.7.1.3 Methanotrophy

$$CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow HCOO^- \rightarrow CO_2$$

Another prevalent process that acts on methane at cold seep sites is bacterial methanotrophy via one of two different biochemical pathways that are utilized by two different classes of methanotrophs.

Both of these pathways for methanotrophy utilize methane monooxygenase (MMO) in order to convert methane into formaldehyde. Type 1 methanogens utilize the ribulose monophosphate pathway (RuMP) to assimilate carbon solely from the formaldehyde previously

formed, and Type 2 methanogens assimilate carbon from a mixture of predominately (50-70%) formaldehyde via the serine pathway and the remainder from fixation of CO_2 via phosphenolpyruvate (PEP) carboxylase. The two different pathways for methane oxidation lead to significantly different fractionations for the two pathways for methane oxidation, with Type 1 methanotrophs having a fractionation of 15-25‰ from methane to biomass and Type 2 methanotrophs having a fractionation of 10-15‰ at 22-24°C with moderate cell densities (Templeton et al. 2006). These fractionations are expected to be much smaller at the temperatures observed in bottom waters of the Gulf of Mexico (4-6°C) and when methane is in limiting concentrations and is completely utilized by the symbiont (no fractionation resulting). Type 2 methanotrophs are present in these cold seeps, but do not play a significant role in this study and will not be addressed further.

Methanotrophy is the process by which the mussel symbionts process available methane. The most depleted methane values in the mussels would be expected from a prevalent source of methane that has not previously been acted upon by methane oxidizers, with the maximum possible depletion in values found in the mussel tissue a result of the initial uptake occurring between a methane pool in the exterior environment into the symbionts. This uptake would coincide with enrichment in the remaining methane pool and the next mussel to encounter that pool of methane would subsequently have a more enriched $\delta^{13}C$ value than the previous mussel. When oxidation of methane is more complete as a percentage of the initial value of the pool, the methane has a more enriched $\delta^{13}C$ value. This is a result of depletion in ^{12}C and a subsequent higher population of ^{13}C within the pool at lower concentrations. This is a result of both limiting concentrations (point source situation with little resupply) and previous processing by methane oxidizers (Barker and Fritz 1981).

1.7.1.4 Photosynthesis

Another source of carbon to cold seeps is carbon derived from photosynthesis in the form of marine snow and particulate organic matter (POM) raining down from the surface waters. At the depths of the sites in this study (450-650 m) this resource is still relatively labile in comparison to most other hydrothermal or cold seep settings which are in much deeper waters (~3200 m) (Olu et al. 2009; Paull et al. 1989). POM is seasonal in nature and the rate of deposition fluctuates greatly with the amount of phytoplankton in the water column. Typical deep slope organisms derive their carbon and nitrogen from both the rain of photosynthetic derived POM that occurs as phytoplankton die or are consumed by zooplankton (Burd and Jackson 2009) and the highly refractory carbon that comprise 0.1-1% of the dry weight of deep sea sediments (Jorgensen and Boetius 2007). Due to an assimilation efficiency of ~10% and the relatively refractory nature of these available sources, turnover rates of microbes associated with the sediments and biomass of larger organisms in the deep sea both tend to be small. The POM $\delta^{13}C$ value for surface waters in the Northern Gulf of Mexico is ~ -21 to -22‰ (Rooker et al. 2006; Wells and Rooker 2009).

1.7.1.5 Chemosynthesis

The $\delta^{13}C$ value for *Beggiatoa* (sulfur oxidizing bacteria) is -31‰ and as such is intermediate in value between POM and both types of methane encountered in Gulf of Mexico cold seep settings (Demopoulos et al. 2010).

1.7.2 Nitrogen

This element is typically used as a trophic level indicator in food webs, with a typical 3.4‰ enrichment with each trophic level added to a food web (Vander Zanden and Rasmussen 2001). The predominant application of $\delta^{15}N$ values in this study will be to identify and address the relative contribution of nitrogen resources at the different cold seep sites and not the delineation of trophic levels within the food web. POM is a source of nitrogen to the bottom waters of the Gulf as well as carbon. The $\delta^{15}N$ of POM in the Gulf of Mexico ranges from 2-8‰ (Rooker et al. 2006; Wells and Rooker 2009). At both sites in this study, there appears to be direct incorporation of ammonium, which leads to a fractionation (-14-27‰) (Hoch et al. 1992) that greatly depletes the $\delta^{15}N$ values of the organisms utilizing it as a nitrogen resource. Mussels, their symbionts, and free living bacteria all have the ability to directly uptake ammonium and nitrate that is available in cold seep settings (Lee and Childress 1994). There is likely significant incorporation of bottom water nitrate in these systems with global values ranging from 5-7‰ (Liu and Kaplan 1989) with regional values in the Gulf of Mexico being close to the global average (B. Fry personal communication). *Beggiatoa* (sulfur oxidizing bacteria) have a measured value of $\delta^{15}N$ of -3‰ in the Northern Gulf of Mexico and as such have an intermediate value between POM, bottom water nitrate, and ammonium utilization (Demopoulos et al. 2010).

1.7.3 Sulfur

$\delta^{34}S$ is typically used as an indicator of an organisms degree of benthic interaction or relative interaction with oxic/anoxic interfaces in estuarine settings (Peterson and Fry 1987). This capability is due to the large degree of depletion that occurs during sulfate reduction that creates a reliable signal that is not confounded or muddled by other metabolic pathways involving sulfur (Canfield 2001). The predominant form of sulfur in oceanic settings is seawater sulfate (SO_4^{2-}) and it has a $\delta^{34}S$ value of 21‰ (Rees et al. 1978). In benthic settings, this sulfate is acted upon by bacterial sulfate reduction, resulting in a cumulative KIE of 30-70‰ (Canfield 2001). This resulting hydrogen sulfide typically has negative $\delta^{34}S$ values that are greatly depleted compared to the $\delta^{34}S$ of seawater sulfate (Fu 1998; Peterson and Fry 1987). If this hydrogen sulfide is then subsequently utilized by sulfur oxidizing bacteria in mats on the surface of the sediments or free living in close proximity to the sediment water interface, then the $\delta^{34}S$ values of the resulting sulfur pool within the sulfur oxidizing bacteria will be extremely depleted due to the fractionation of 10-18‰ associated with the oxidation of hydrogen sulfide (Dale et al. 2009). In cold seep settings, sulfur oxidizing bacteria are an important sulfur source that carry a readily identifiable extremely depleted $\delta^{34}S$ signal.

1.8 Three Isotopes, Three Values, One Source

In these studies, modeling CNS isotopes requires the use of sources that have values for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ which are then incorporated into the organisms feeding or utilizing the sources. For example, $\delta^{13}C$ values of most pelagic organisms approach that of marine POM (-22‰) (Peterson and Fry 1987), with their $\delta^{15}N$ values indicating position in trophic structure relative to their food sources, and $\delta^{34}S$ values indicating the usage of marine sulfate (21.8‰) as their primary sulfur source (Rees et al. 1978). The requirement for three separate isotope values leads to a fair amount of interpretation about the values for $\delta^{15}N$ and $\delta^{34}S$ for several of the sources due to a lack of established measurement values for basal resources. Problems such as this are inherent in systems that lack extensive sampling due to the expense and the relatively new abilities of isotope techniques. Concerns about the accuracy of basal resource values are valid and must be kept in perspective, but should not completely invalidate the insights shown through the use of CNS mixing models.

1.9 Objectives

This project attempts to identify and explain the variations in carbon, nitrogen and sulfur stable isotopes in order to gain a better understanding of the processes occurring within these chemosynthetic communities. These measurements should allow for useful inferences about the extent of utilization and variation of multiple unique sources that are available in these settings. The scope of this project is to provide spatial detail within mussel beds using a sampling design with paired interior and edge sites within the existing cold seep sites to gain insight on the heterogeneity that seems to be inherent within cold seep ecosystems and their associated populations. Unlike previous studies which have utilized many species with few replicates in various settings, this study focuses on the chemosynthetic mussel *Bathymodiolus childressi*, with a large number of replicates in multiple sites across multiple temporal settings.

Stated as questions:

1. Do the means and variances of stable isotopes in mussel soft tissues indicate steady state or changing conditions at the two sites examined?

2. Does the carbon isotope composition of mussel shells provide similar information about populations which might be used when tissue is not available?

2 Chapter 2: Site-Specific Incorporation of Methane-Derived Respiratory Carbon in the Shells of the Seep Mussel *Bathymodiolus childressi*.

2.1 Introduction

2.1.1 Background

Methane seeps on the upper continental slope (400 m to 1000 m depth) of the Northern Gulf of Mexico appear to be transient habitats within larger seafloor areas of persistent hydrocarbon migration upward through the seafloor (Macdonald et al. 2003; H. H. Roberts and Carney 1997). As local seep habitats progress from an initial fluid to a mineral phase there is an accompanying colonization and change in associated biotic communities (Bergquist et al. 2005; Bergquist et al. 2004a). Among the apparent stages of seep community progression is that of dead or dying systems. The former is marked by an abundance of bivalve shell debris, and the latter is marked by similar debris accompanied by a few living mollusks.

Dead and dying seep assemblages may reflect cessation or reduction of methane flux, if the dominant mollusk is fully or partially dependent on methanotrophic symbionts (Callender and Powell 1997). Other contributing factors might be failure of recolonization following predation or disease (Zielinski et al. 2009). In the case of a decrease in methane flux, it can be expected that shell material may retain some record of the event so long as the organisms survive, and continue to deposit shell during the decline and possibly after the cessation of methane seepage.

The two objectives of the work reported herein are to first assess the feasibility of detecting a $\delta^{13}C$ methane signal in shell from live sampled *Bathymodiolus childressi* specimens over a range of size, and second to see if such information could be useful in assessing spatial differences in the availability of seep methane experienced by the biota. The feasibility of this approach was suggested by work on the deposition of respiratory carbon in aquatic molluscan calcium carbonate. Aquatic mussel shells have been shown to be comprised predominately of seawater dissolved inorganic carbon (DIC) with a small contribution of respiratory carbon (~10%) from the animal. It has been proposed that an increase in metabolic activity can contribute to a higher portion of respiratory carbon being incoporated into the shell thus leaving a permanent record of metabolic activity preserved in the shell (Mcconnaughey et al. 1997; Mcconnaughey and Gillikin 2008).

2.1.2 Shell Introduction

Bathymodiolus shell is composed of four shell layers, an outer fibrous prismatic calcite layer, an inner nacreous aragonite layer, an intermediate nacreous layer that is composed of aragonite and an overlying periostracal layer that is composed of protein (Kennish et al. 1998). The shell of a related hydrothermal vent species is composed predominately of calcite (72-95%) with the remainder being aragonite (Demina et al. 2012). Between the shell and the mantle of

the mussel there is an extrapallial fluid (EPF) filled cavity where calcification occurs. The EPF is consistently supersaturated with calcium carbonate which is achieved through the alkalization of the fluid. Alkalization happens through the use of an ion transporter Ca^{2+} ATPase which pumps protons away from the site of calcification and creates an increase in pH of 0.5 units. There is a resulting shift in the predominant carbonate species from HCO_3^- to $CO_3^=$ due to deprotonation. This process results in a 4-6 fold increase in the concentration of $CO_3^=$ in the EPF versus the surrounding tissues (Mcconnaughey and Gillikin 2008) and results in a depletion of HCO_3 in the surrounding tissues that sets up a diffusion gradient. CO_2 is constantly entering the tissues and the EPF in order to maintain the supersaturation of $CO_3^=$ that is conducive to calcification. Kinetic fractionation effects associated with the deposition of $CaCO_3$ are small in these cold seep sites. The EPF is a calcification physiology does not require the transport of HCO_3^- and $CO_3^=$ across thin membranes as is the case for corals and foraminifera. As a result, mussels do not demonstrate the larger 6-22‰ depletion of $\delta^{13}C$ commonly associated with coral and foraminifera autotrophic calcification physiologies (Mcconnaughey et al. 1997).

2.1.3 Shell and Metabolism

Aquatic mollusk shells contain a record of two pools, respiratory carbon produced by the animal and dissolved inorganic carbon (DIC) from the surrounding seawater, which is the predominate source of CO_2 for shell formation. This is in stark contrast to air breathing animals where the predominate carbon source of deposited hard parts (bone, shell) is respiratory carbon and results in these hard parts being isotopically closely representative of the $\delta^{13}C$ of their diet, although offset by consistent isotope effects. The difference in predominant carbon source for calcification between air breathing and water breathing animals is a result of atmospheric CO_2:O_2 ratios being around thirty times lower than in aquatic settings. Air breathing animals having significantly less intake of CO_2 as they uptake O_2 (Mcconnaughey et al. 1997). Aquatic animals incorporate a small portion of their respiratory carbon along with seawater DIC due to the increased DIC available in the seawater. The percentage of respiratory carbon incorporated into hard parts range from 7-15% in aquatic mollusks, ~30% in fish otoliths and up to 90-95% in birds, mammals, and land snails.

When food is abundant, metabolic rates are higher, and there is an elevated amount of respiratory carbon within the EPF for shell deposition. Conversely, when food resources are low, metabolic rates slow and less respiratory carbon is available for deposition. The expectation is that the isotopic composition of the shell might reflect the availability of resources (Mcconnaughey and Gillikin 2008).

2.1.4 Calculation of Inclusion of Respiratory Carbon Into Shells

The fraction of respiratory carbon incorporated into the shell (C_m) is calculated using the proportional mixing formula from McConnaughey and colleagues (Mcconnaughey et al. 1997) which calculates C_m as a function of the respective contributions from respiratory sources obtained using the $\delta^{13}C$ mussel adductor tissue values detailed in Chapter 3 and the dissolved inorganic carbon pool.

The formula used for this calculation in this study is:

$$\delta^{13}C_{shell} - \Delta = (\delta^{13}C_{tissue} * C_m) + (\delta^{13}C_{DIC} * (1 - C_m))$$

Where:

$\delta^{13}C_{shell}$ (‰) is the measured value of the shell for the individual mussels.

Δ is a fractionation correction for the $\delta^{13}C$ of the two crystalline forms of $CaCO_3$, calcite and aragonite and has $\delta^{13}C$ value of 2.1‰ for the Brine Pool and 2.3‰ for Bush Hill. These fractionation corrections are based 35/65 (% calcite/% aragonite) at the Brine Pool site and a 25/75 at the Bush Hill site (D. Feng, personal communication and unpublished data). These calcite/aragonite compositions were obtained through X-ray diffraction on the shells of a subset of the populations at both sites. Mussels at both sites have a higher composition of aragonite than those found for mytilid mussel shells by Demina and colleagues (Demina et al. 2012) (8-30 /70-92). The corrections used here weigh the fractionation factors for $\delta^{13}C$ associated with the formation of aragonite (2.7‰) and calcite (1‰) (Romanek et al. 1992).

$\delta^{13}C_{tissue}$ (‰) is the measured adductor tissue value for the individual mussel and serves as the source value of the respiratory carbon incorporated into the shell.

$\delta^{13}C_{DIC}$ (‰) is the value of the surrounding dissolved inorganic carbon pool in the seawater. This was measured in a previous study to be 0.69‰ (Fu 1998).

C_m is the proportional contribution of metabolic carbon assuming 100% contribution from the two pools.

The formula was solved for C_m and converted for utility into a percentage by multiplying by 100. C_m was subsequently evaluated against Ln(half shell volume (HSV)) and also between and within mussels beds using paired T tests ($\alpha = 0.05$) and ANOVA for temporal results with Tukey's studentized range ($\alpha = 0.05$) performed as a post hoc analysis.

2.1.5 Growth Correction

A trend of increasing depletion of $\delta^{13}C$ of the shell with increasing volume has been observed in several epifaunal and infaunal bivalves (Gillikin et al. 2007). This trend has been attributed to the increase in incorporation of metabolic carbon due to an increase in metabolic CO_2 production that is larger than the demand for CO_2 for calcification, resulting in more metabolic carbon in the internal DIC pool as the shell is more slowly precipitated as the organism grows (Lorrain et al. 2004). This correction factor was calculated by regressing $\delta^{13}C_{shell}$ against Ln(HSV) for both sites. Slopes were not significantly different ($p=0.325$). A common intercept was established by applying a translation of 7.1 ‰ to the Brine Pool site. A second regression of the translated $\delta^{13}C_{shell}$ values was carried out ($b=-0.685$, $R^2=0.14$). Size effects were eliminated by subtraction of predicted values based on the regression. The

magnitude of the correction ranged from 0.2% for a shell with a volume of 1.1 cm^3 to 5.9% correction for a shell with a volume of 35.9 cm^3.

2.2 Methods

2.2.1 Sampling

This study made use of archived specimens. The initial field sampling began in 1989 by Robert S. Carney of LSU and was intended to determine spatial differences in species composition and trophic linkages in mussel dominated seep assemblages (R. S. Carney 1994; Macavoy et al. 2003). Shell analysis was not included in the suite of original work. Mussels were sampled using the submersible Johson SeaLink, preserved in 5% seawater formalin and maintained in archival storage following initial analyses. Analyses reported herein are based on specimens for two sites of hydrocarbon seepage (Fig. 1.1), Brine Pool NR-1 in Green Canyon Lease Block 233 (GC 233) sampled in 1989 and 1991 with a more recent resampling in 2006, and Bush Hill (GC 185) sampled in 1991 and 1992.

Bush Hill is an expulsion feature located along a seep-prone fault flanking a large salt diapir with isolated mussel clumps scattered over a topographically complex area of sediment, exposed authigenic carbonates and methane hydrate mounds (Macdonald et al. 2003). Methane flux at the seafloor appears to be highly localized and methane originated from an underlying hydrate reservoir. Brine Pool NR-1 is a lower-relief feature in an area of extensive slope failures and has been characterized as a quiescent mud volcano (Joye et al. 2009). Methane flux appears to come from the pool which is surrounded by a continuous bed of mussels.

2.2.2 Preparation of Samples

In 2010, archived mussel specimens were opened and washed in a deionized (DI) bath for up to 24 hours to dilute and remove seawater salt, sulfate, and excess formaldehyde. The mussels were then dissected, and tissue and shell dried at 60°C for a minimum of 12 hours until completely dry. Tissue samples were taken from the adductor muscle. Shell samples were broken from the outer 1 cm edge of a single valve incorporating all calcified layers. The adductor muscle was processed using a WIG-L-BUG (Dentsply International) into as fine a powder as possible and loaded for CNS analysis. The outer periostracum organic layer was removed from the dried shell sample. The bare shell was ground into a fine powder, treated with sodium hypochlorite bleach for 12 hours to remove organic material, rinsed copiously with DI, dried again and loaded for analysis.

2.2.3 Measurement of Half Shell Volume

Since carbon is respired throughout the volume of living tissue, a volumetric estimate of shell size was developed based on the relationship between volume and height found in a subsample of 19 shells (Rodhouse 1977). Shell length and height were measured to the nearest millimeter. Length was measured along the longest axis and height was measured as the maximum vertical length when the valve was placed face down on a flat surface. Mussel half

shell volume (HSV) was measured using 450-500 micrometer glass beads (Sigma G9268) to completely fill a single valve and then weighed. A vessel was filled with glass beads and then water in order to establish a conversion factor (0.57) from glass bead weight into water weight. The water weight of the measured volume was then regressed against the shell height (mm) of the selected mussels. The utility of shell length to estimate HSV was first examined by regression (R^2= 0.79, HSV=1.4249e^(0.314(length)). Shell height, however, proved a better basis for estimation (R^2=0.96). HSV= 0.4377e^(0.2144(height)), was then used in order to calculate HSV(cm^3) for each individual mussel. The subsample of the population (n= 19) that were used to establish this relationship ranged across a wide range of HSV (1.4-68.8 cm^3). A potential limitation of using HSV is that the tissue volume can change independently of the shell volume. The ratio of the two components varies seasonally in many species of mytilids (Bayne 1976). This thesis does not address issues of seasonality that are likely to affect both shell volume and metabolism.

2.2.4 Isotopic Analysis

Mussel tissue samples were processed for CNS isotope analysis using the methods described in B. Fry (2007) reporting $\delta^{13}C$ values versus PeeDee Belemnite, $\delta^{15}N$ values versus atmospheric nitrogen, and $\delta^{34}S$ values versus Canyon Diablo Troilite. The 95% confidence level for this sampling is 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$, and 0.4‰ for $\delta^{34}S$. Laboratory check standards were interspersed with a range of weights within each run of 27 samples in order to ensure consistency within and between the sample runs. Twenty two standards and duplicates were included in each run of 49 total analyses. Shell samples were run in the same manner as tissue samples, but only carbon isotope values were examined for shells. Sulfur and nitrogen amounts were minimal and noisy, reflecting the small amount of residual organic matter remaining in the shell after bleach treatment.

2.2.5 Formalin

Formalin is a saturated aqueous solution of formaldehyde which is used for fixation and long-term storage of biological specimens. Fixation hardens tissues without distortion and is caused by binding and cross linking functional groups associated with protein, glycoproteins, and nucleic acids (Fox et al. 1985). These same reactions make formalin a powerful biocide which prevents decay of archived specimens and as such it has been commonly used in past ecological studies at 5% to 10% diluted with seawater. Since the formaldehyde binds with tissues it provides a complication for stable isotope analysis, with the addition of more depleted carbon obscuring the original materials $\delta^{13}C$ value. The depleted $\delta^{13}C$ value for the fixative depends on the source of carbon during manufacture and can be variable both in $\delta^{13}C$ and the incorporation rate across tissue types and species (Lau et al. 2012; Sweeting et al. 2004). Variability in incorporation rates is due to differences in protein and lipid contents of tissues. Magnitudes of the depletion associated with formalin fixation for $\delta^{13}C$ range from -0.8‰ to -2‰ (Edwards et al. 2002; Sweeting et al. 2004) with the largest depletions occurring with initial exposure and becoming less depleted as storage time increased. The previously measured $\delta^{13}C$ values of

between -38‰ and -52.5‰ for formalin from multiple manufacturers (Edwards et al. 2002) fall between the ranges observed for the muscle tissues in this study (-36.6 to -75‰) and could impart either a depletion or enrichment effect depending on the tissue values for the sites with an enrichment expected at the Brine Pool site and a small depletion or enrichment expected at the Bush Hill site.

In this study, Mytilid mussels from a local market were preserved using a subsample of the formalin that was used to preserve the cold seep mussels and resulted in a depletion of -2.6‰ ± 0.09 SD (n=6) (Table 2.1). Assuming a 15% incorporation rate, the calculated value for the $\delta^{13}C$ of formalin is -41.7‰ which is within the range of literature values previously mentioned. Using this value for $\delta^{13}C$ of formalin and assuming the same incorporation rate of 15% for formalin into cold seep adductor muscle, the calculated differences for $\delta^{13}C$ tissue at the Brine Pool range from a depletion of 1.4 to 5.9‰ that result in a reduction to C_m of 2.8 to 7.2% between calculations for C_m with and without formalin corrections. For Bush Hill, the differences in $\delta^{13}C$ range from a depletion of 0.8‰ to an enrichment of 0.9‰ resulting in a 0.8 to 6% reduction in calculated C_m values. Fixation effects will be larger for the Brine Pool site than the Bush Hill site and could significantly affect the calculations for C_m between sites by resulting in an underestimate for the amount of incorporation at the Brine Pool site than at the Bush Hill site. The mussels from all samplings excluding the 2006 year Brine Pool (which were frozen), are corrected using a 15% incorporation rate and a -41.7‰ for $\delta^{13}C$ for formalin.

Table 2.1: Mytilid mussels divided into treated with formalin (60 days) and control portions in order to establish the formalin effects on $\delta^{15}N$, $\delta^{13}C$, and C/N ratios for this study using the same formalin that the cold seep mussels were stored in. The net effects were an increase in $\delta^{15}N$ of 0.3‰, a decrease in $\delta^{13}C$ of 2.6‰, and no effect on C/N ratios. Δ is the difference between formalin treated and untreated sample.

Samples	$\delta^{15}N$ (‰)	$\Delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{13}C$ (‰)	C/N	ΔC/N
1	9.0		-19.9		3.9	
1 with Formalin	9.1	0.1	-22.6	-2.6	4.0	0.2
2	9.2		-20.0		4.0	
2 with Formalin	9.4	0.2	-22.6	-2.5	4.0	0.0
3	8.6		-20.2		4.2	
3 with Formalin	9.0	0.4	-22.7	-2.5	4.0	-0.2
4	9.0		-19.9		4.1	
4 with Formalin	9.0	0.0	-22.6	-2.8	4.0	-0.1
5	8.9		-20.1		4.0	
5 with Formalin	9.2	0.4	-22.7	-2.6	3.9	-0.1
6	8.5		-20.2		3.9	
6 with Formalin	9.0	0.5	-22.7	-2.5	4.0	0.1
Average Δ for All Samples		**0.3**		**-2.6**		**0.0**

2.3 Results

2.3.1 Data Analysis

Sample size across sites was n=154 individuals. At the Brine Pool (n=92), there were sufficient samples to compare among years (1989 n=31, 1991 n=16, 2006 n=45) using an ANOVA analysis and within mussel bed (North n=43, South n=49) (Interior n=27, Edge n=19) using paired T-tests. At Bush Hill (n=62), there were only sufficient samples among all sites sampled (Bed 1 (n=25), Bed 2 (n=17), Bed 1a (n=20)) to compare between year samplings at Bed 1 (1991 n=15, 1992 n=10) and within mussel bed (Interior n=10, Edge=5) using Paired T-tests (α=0.05). Paired T-tests (α=0.05) were used to analyze the differences in C_m between whole site populations and then again while blocking for size in the range of 0 to 10, 10 to 20, and 20+ mm^3 and yielded statistically significant results (Fig. 2.1).

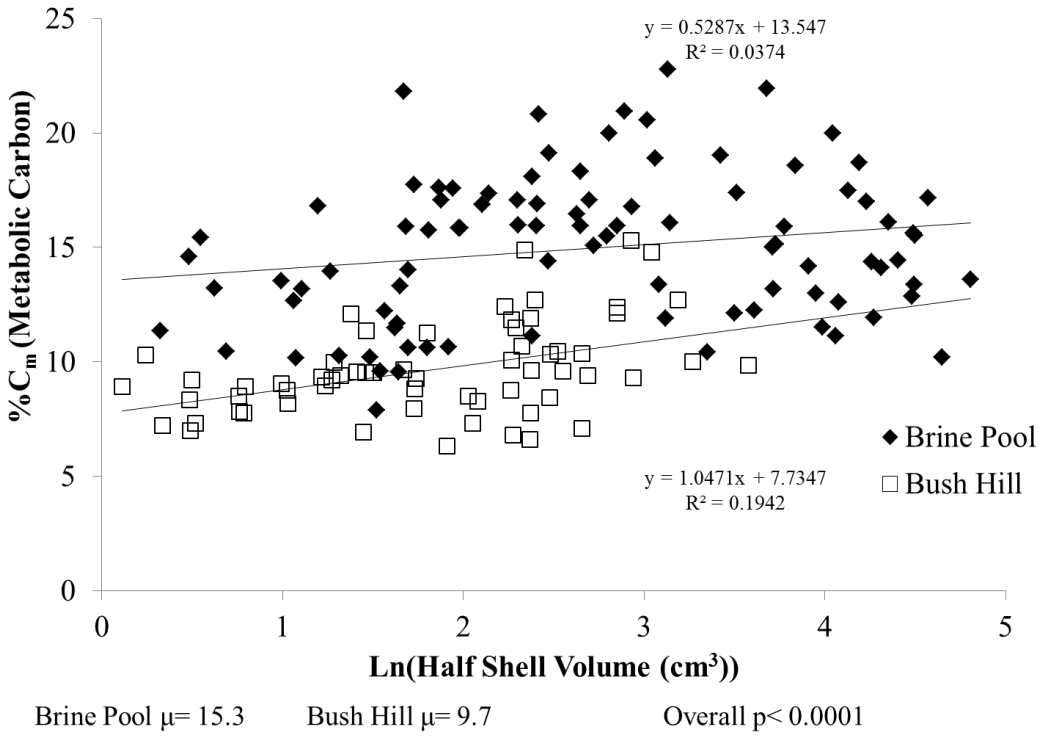

Figure 2.1: C_m between sites versus natural log of Half Shell Volume. Using an ANCOVA analysis between the two sites the slopes of the two regressions are not significantly different (p=0.6405), but the intercepts are (p<0.0001, BP 13.55, BH 7.7). The higher mean for the Brine Pool site of 15.3 ± 3.3 indicating higher metabolism for this site and therefore higher resource availability than at the Bush Hill site with a mean of 9.7 ± 2. At Bush Hill, the combination of smaller sizes and reduced C_m indicate a site with relatively more resource limitation.

2.3.2 Shell Values

2.3.2.1 Size Effect on C_m at Each Site

Separate regression of C_m versus HSV at Brine Pool and Bush Hill revealed significant ($p<0.0086$, $p<0.0001$), but relatively small, linear positive relationships between Ln(HSV) and C_m at each site that are not significantly different from one another ($p=0.1977$). The intercepts (13.55, 7.7) at the two sites were significantly different ($p<0.0001$) (Fig 2.1). A two sample T-test of C_m between the sites was significantly different ($p<0.0001$). Higher mean C_m at the Brine Pool ($n=92$) site of 15.3 ± 3.3 versus the mean at Bush Hill ($n=62$) of 9.7 ± 2 indicates higher availability of food for the population at the Brine Pool. An ANOVA with Tukey's studentized range performed as a post hoc test ($\alpha= 0.05$) performed on the Brine Pool site on C_m for groupings of HSV from 0 to 10 cm^3, 10 to 20 cm^3, and 20+ cm^3 resulted in significant differences between the 0-10 cm^3 ($n=36$) grouping with the mean being 13.7 ± 3.2 and the 10 to 20 cm^3 ($n=18$) with the mean being 16.9 ± 2.4 and also between the 0 to 10cm^3 grouping and the 20+ cm^3 ($n=38$) grouping with a mean of 16 ± 3.2 (Table 2.2). A second ANOVA performed on the Bush Hill site resulted in significant differences between 0 to 10 cm^3 ($n=40$) with a mean of 9 ± 1.5 and 10 to 20 cm^3 ($n=18$) with a mean of 10.5 ± 2.4 and between 0 to 10 cm^3 and 20+ cm^3 ($n=4$) with a mean of 11.8 ± 2.4 (Fig. 2.2).

Table 2.2: C_m for both sites grouped on Half Shell Volume (HSV). Groupings for both sites are 0 to 10, 10 to 20, 20+, and All for all size groups pooled for each site. Using Tukey's studentized range as a post hoc test, there are significant differences ($\alpha=0.05$) between the 0 to 10 and 20+ groups and the 10 to 20 and 20+ groups at both sites. A paired T-test between the sites with all size groupings pooled is significantly different between sites ($p<0.0001$). Paired T-tests between the sites for each size group are significant with 0 to 10 ($p<0.0001$), 10 to 20 ($p<0.0001$), and 20+ ($p=0.0139$).

Site	Volume (cm^3)	Average C_m	Standard Deviation	n	Standard Error
Brine Pool	0 to 10	13.7	3.2	36	0.5
Brine Pool	10 to 20	16.9	2.4	18	0.6
Brine Pool	20+	16.0	3.2	38	0.5
Brine Pool	All	15.3	3.3	92	0.3
Bush Hill	0 to 10	9.0	1.5	40	0.2
Bush Hill	10 to 20	10.5	2.4	18	0.6
Bush Hill	20+	11.8	2.4	4	1.2
Bush Hill	All	9.7	2.0	62	0.3

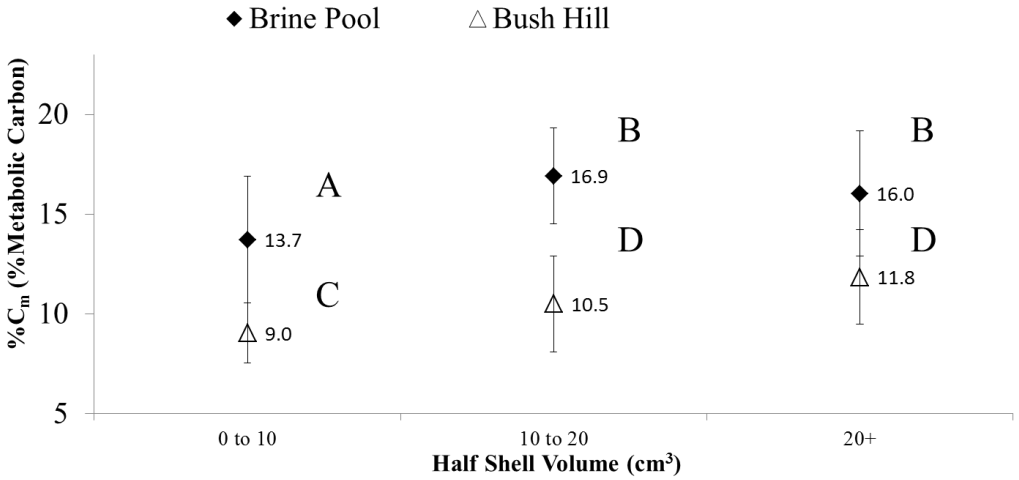

Figure 2.2: C_m Between Sites Grouped on Half Shell Volume. ANOVA analysis with Tukey's studentized range (α=0.05) for the groupings of the mussels within sites by ranges of HSV.

2.3.3 Size Effect on C_m Between Sites

When these samples are grouped on ranges of HSV and compared again between the Brine Pool and Bush Hill, there was a significant difference ($p<0.0001$) (Fig. 2.2) between the Bush Hill and Brine Pool sites for a mussel HSV of 0 to 10 cm^3 with the mean at the Brine Pool (n=36) of 13.7 ± 3.2 and at Bush Hill for 0 to10 cm^3 (n=40) of 9 ± 1.5 (Table 2.2). For a mussel HSV of 10 to 20 cm^3, there was a significant difference ($p<0.0001$) between the two sites with the mean at the Brine Pool (n=18) being 16.9 ± 2.4 and the mean at Bush Hill (n=18) being 10.5 ± 2.4. For a mussel HSV of 20+ cm^3, there was a significant difference ($p< 0.0139$) between the two sites with the mean at the Brine Pool (n=38) being 16 ± 3.2 and the mean at Bush Hill (n=4) being 11.8 ± 2.4 (Fig.2.2). There is a significant difference in the incorporation of metabolic carbon between the sites for all tested size ranges, with the Brine Pool having significantly increased C_m.

2.3.4 Spatial Effects

T tests performed on within mussel bed differences at the Brine Pool (North (n=43) versus South (n=49)) revealed a statistically significant difference ($p< 0.004$) (Table 2.3) with the South having a larger mean of 15.9 ± 3.7 than the North of 14 ± 2.3 with all sample years pooled. In individual year samplings for North versus South (Table 2.3), the 1989 and 1991 samplings were not significant, but the 2006 sampling was significant ($p<0.004$) with the South (n=34) having a larger C_m of 17.4 ± 3 than the North (n=11) site with a mean of 15 ± 1.2. There was no such North and South structure at the Bush Hill site. The further detailed Interior and Edge sampling design for both the Brine Pool and Bush Hill yielded only one significant result ($p=0.017$) in the Bush Hill 1991 sampling with a larger C_m at the Interior (n=10) of 8.9 ± 1.4 and a smaller C_m of 7.1 ± 0.7 at the Edge (n=5).

Table 2.3: Spatial Effects on C_m. Within mussel bed averages for spatial sampling. All refers to pooled samplings of 1989, 1991 and 2006 specimens for both North and South bed designations.

Site	Year	Mussel Bed	Average C_m	Standard Deviation	n	Standard Error
Brine Pool	All	South	15.9	3.7	49	0.5
Brine Pool	All	North	14.0	2.3	43	0.3
Brine Pool	1989	North	13.6	2.7	20	0.6
Brine Pool	1989	South	12.3	2.9	11	0.9
Brine Pool	1991	North	13.7	2.1	12	0.6
Brine Pool	1991	South	12.8	3.2	4	1.6
Brine Pool	2006	North	15.0	1.2	11	0.4
Brine Pool	2006	South	17.4	3.0	34	0.5

2.3.5 Temporal Effects

An ANOVA analysis with a Tukey's studentized range performed as a post hoc test (α=0.05) for comparison between years was used in order to compare C_m between 1989, 1991 and 2006 samplings at the Brine Pool (n=92) with a statistically significant difference found between sample years ($p<0.0001$) (Fig 2.3 A.). At the Brine pool site in the 1989 sampling (n=31), the mean was 13.1 ± 2.8 and was significantly different than the 2006 sampling. In the 1991 sampling (n=16), the mean was 13.5 ± 2.3 and was significantly different from the 2006 sampling, but not from the 1989 sampling (Table 2.4). In the 2006 sampling (n=45), the mean was 16.8 % ± 2.8. A paired T test between Bush Hill mussels collected at Bed 1 in 1991 and 1992 yielded a statistically significant difference ($p<0.0001$) with the mean in 1991 (n=15) being 8.3 ± 1.5 and the mean in 1992 (n=10) being 12 ± 2.2 (Fig 2.3 B.). There were increases in C_m between the 1989 and 2006 and the 1991 and 2006 samplings for the Brine Pool and an increase in C_m at the Bush Hill site between the 1991 and 1992 samplings.

Table 2.4: Temporal Effects on C_m. All refers to the usage of all sampling beds for the Brine Pool, since repeated samplings did not occur at Bush Hill except for Bed 1.

Site	Mussel Bed	Year	Average C_m	Standard Deviation	n	Standard Error
Brine Pool	All	1989	13.1	2.8	31	0.5
Brine Pool	All	1991	13.5	2.3	16	0.6
Brine Pool	All	2006	16.8	2.8	45	0.4
Bush Hill	Bed 1	1991	8.3	1.5	15	0.4
Bush Hill	Bed 1	1992	12.0	2.2	10	0.7

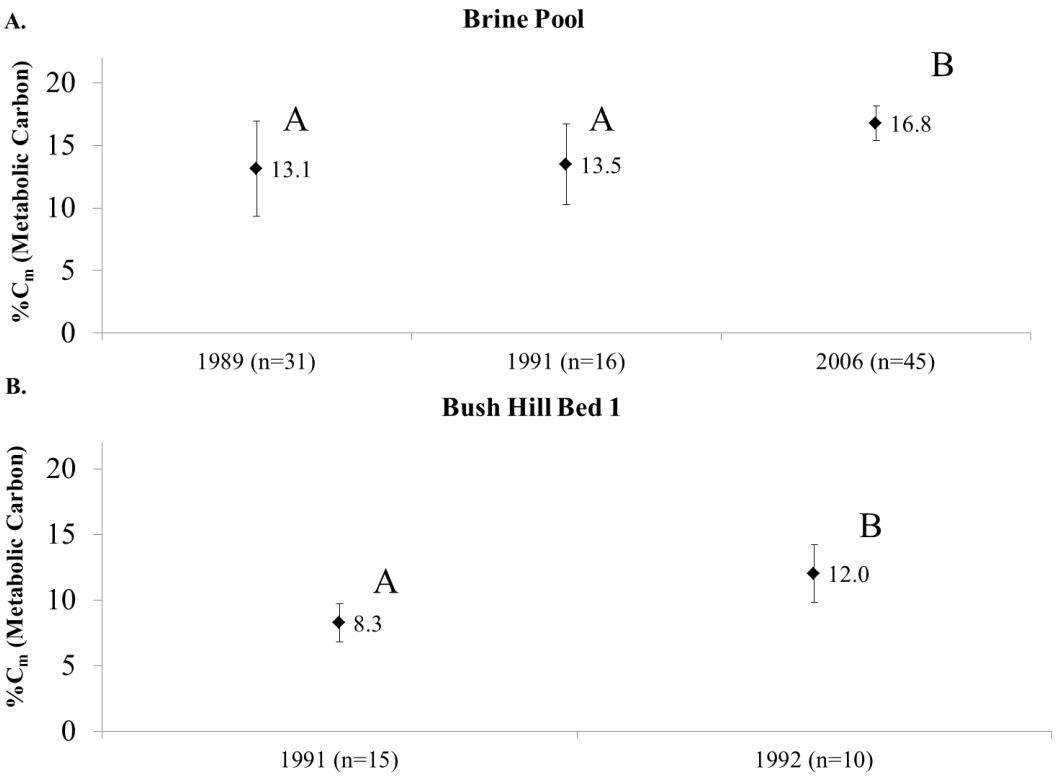

Figure 2.3: Temporal effects on C_m. A) ANOVA analysis for Brine Pool site with Tukey's studentized range as a post hoc test showing an increase in C_m in 2006 versus 1989 and 1991 indicating significant change in C_m across a decadal time scale. B) A two sample T-test between 1991 and 1992 at Bush Hill Bed 1 was significantly different ($p<0.0001$) indicating a significant increase in C_m on a much shorter time scale between 1991 and 1992.

2.4 Discussion

2.4.1 Cold Seep C_m versus Estuarine C_m

Previous work establishing C_m as an indicator in mollusks has been performed on estuarine or nearshore species. This is the first study of C_m performed in a large population of cold seep mussels and as such is aiming towards a proof of concept in a substantially different ecosystem. In the case of *Bathymodiolus childressi*, a cold seep mussel, the depleted nature of the respiratory $\delta^{13}C$ from methane (~ 40-60‰ more depleted than seawater DIC depending on methane type) utilized as a predominant food resource is quite distinct from seawater $\delta^{13}C$ DIC (0.69‰ $\delta^{13}C$) (Fig. 2.5). This leaves the $\delta^{13}C_{shell}$ values of these cold seep mussels significantly more depleted and with a wider range (2-3 times more depleted depending on methane source) than estuarine aquatic mollusks that feed on only phytodetritus (~20‰ more depleted than seawater DIC) as a food source. Assuming a seawater $\delta^{13}C$ DIC value of 0.69‰, calculating a 1% increase in the incorporation of respiratory carbon into the shell results in a depletion of 0.207‰ per 1% increase using the $\delta^{13}C$ value for phytoplankton (-22‰), a 0.407‰ per 1%

increase for the $\delta^{13}C$ value for thermogenic methane (-40‰), and a 0.607‰ per 1% increase using the $\delta^{13}C$ value for biogenic methane (-60‰). The increase of the scale of differences imparted by the utilization of increasingly depleted food resources allows for a greater degree of confidence in the observed rates of C_m in cold seep mussels when compared to estuarine mussels.

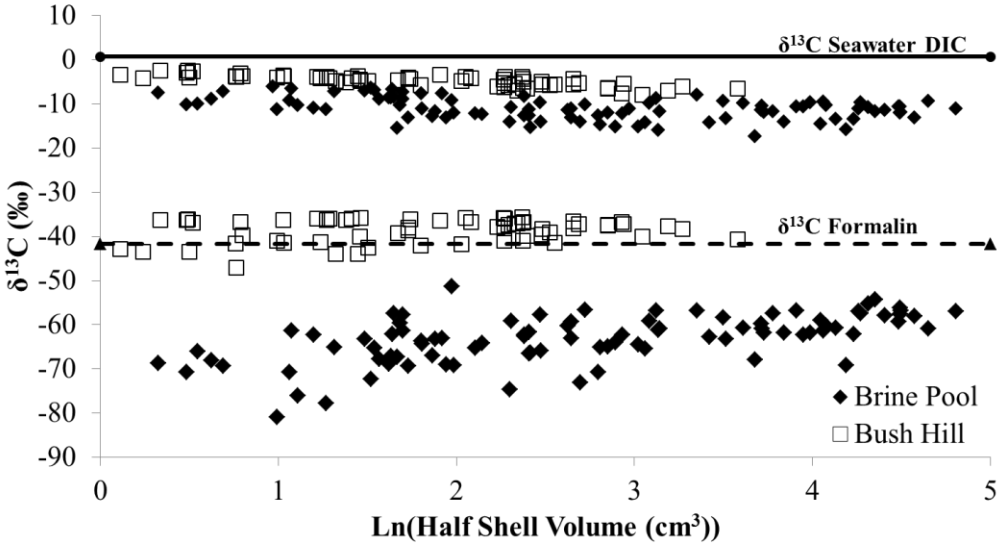

Figure 2.4: $\delta^{13}C$ of Mussel Soft Tissue, Shell, Formalin, and Seawater Dissolved Organic Carbon for Both Sites. $\delta^{13}C$ values for mussel shell and tissue with lines indicating the $\delta^{13}C$ value of seawater DIC and $\delta^{13}C$ value of formalin. The very depleted -(82-75‰) values for small mussel tissue at the Brine Pool site indicate reliance on a more depleted methanogenesis derived methane. The broad range in tissue values as mussels increase in size at the Brine Pool site indicate utilization of other resources than methane and an increased reliance on filter feeding of less depleted materials (phytoplankton, sulfur oxidizing bacteria) in addition to methane.

2.4.2 Resource Differences for Cold Seep Mussels

The large (~20‰) depletion between the methane resources at each cold seep site result in a clear delineation of resource differences between the two cold seep sites in $\delta^{13}C_{shell}$, with the depletion being 33% greater for biogenic methane than thermogenic methane. This method could be used in the future for disarticulated shells in order to identify to within a range the $\delta^{13}C$ of the methane available at the site at the time of active seepage. There are a wide range of observed values for $\delta^{13}C_{Tissue}$ that affect the calculation of C_m and determinations using only disarticulated shell $\delta^{13}C$ values and an assumed value for mussel tissue without confirmation via a proxy for $\delta^{13}C_{Tissue}$ will be speculative at best without considerable population sample size, but may be able to provide more information about site history than otherwise previously available. The estimation, however, of this process would be greatly improved with the inclusion of a proxy for $\delta^{13}C_{tissue}$. Shell protein (Marie et al. 2011) might serve this purpose as a proxy and can

be obtained through shell acidification and subsequent freeze drying. The amounts of sample retrieved are very small and this leaves little room for replication and requires the complete destruction of the shell.

2.4.3 $\delta^{13}C$ DIC of Bottom Water

This study uses bottom water values of $\delta^{13}C_{DIC}$ of 0.69‰ for both sites. This was done due to the lack of measured $\delta^{13}C_{DIC}$ for the sampling time period, and the range of variability of bottom water DIC typically being fairly small for most continental margin settings. The unique geological features present near to these cold-seep sites may render this assumption invalid, but the range of the difference in $\delta^{13}C_{DIC}$ is likely to be 1 to 2‰ and as such should not have a large enough impact as to render the results of this study invalid. The $\delta^{13}C_{DIC}$ of the bottom water at cold seep sites is less variable than estuarine settings since there is no freshwater influence or mixing of vastly different water bodies to affect the $\delta^{13}C_{DIC}$ values. As with estuarine settings, the $\delta^{13}C_{DIC}$ quickly becomes increasingly depleted in the pore waters under the site (2 cm, -11.1‰ (Fu 1998)), so there is potential for incorporation of a substantially more depleted signal into the shell. *Bathymodiolus childressi* are in beds above the sediment/water interface and are actively pumping water across their gills in order to respire. This configuration makes it less likely for a large amount of incorporation of depleted pore water DIC than in mussel species that burrow into the underlying sediments. Because the $\delta^{13}C$ of the pore water is very depleted compared to the seawater DIC, it is reassuring that at the Bush Hill site the $\delta^{13}C_{shell}$ for mussels less than 3 cm^3 is greater than -2‰, indicating that the smallest mussels with the highest growth rate and quickest tissue carbon turnover have a more positive $\delta^{13}C_{shell}$ than what the end result of a seawater DIC/pore water DIC mixture and metabolic carbon from thermogenic methane would be. There may be some incorporation of pore water in these mussels, but it is likely to be inconsistent in nature and happen as a result of episodic conditions and not across lengthy periods of time. Unfortunately, no data exists on the long term nature of site $\delta^{13}C_{DIC}$ at the mussel beds across time for these samplings, so little can be done to correct for this variable.

2.4.4 Metabolism and Respiration across Ontogeny

Bathymodiolin mussels, like many other animals, expend a great deal of energy for growth at small sizes and as they age expend relatively less energy for growth, with an increased use of energy for reproduction and feeding as a portion of total metabolism (Sukhotin and Flyachinskaya 2009; Thompson and Bayne 1974). Relative respiration rates also drop as a function of both tissue weight and age, but the turnover-time of the carbon pool in the organism also increases as a function with volume. These opposing trends regulate the amount of respiratory carbon incorporated into the shell as an organism grows. When applied to the Brine Pool site, it appears that there is less availability of resources for mussels in the 0-4 cm^3 range due to reduced values for C_m (Fig. 2.2). This may be a function of competition with other larger mussels, leaving the smaller mussels in a less opportune position utilizing a larger portion of more depleted methane sourced from methanogenesis from CO_2. This unique methane source leaves the $\delta^{13}C$ of their tissues more depleted (-65-75‰) than the methane value at the brine

pool site (-64‰) (Sassen et al. 1999) and not as readily able to compete for space with access to higher concentrations of ambient methane (Fig. 2.3). This competition effect seems to abate as the mussel's volume reaches around 7 $cm^3 - 20$ cm^3, where the maximum values of C_m are observed (Fig. 2.2). This is likely due to a combination of larger gill surface area for methanotrophic symbionts and increased growth rates as the mussels are able to compete more effectively for access to food. Greater than 20 cm^3, the values for C_m decline overall as would be expected for large mussels as respiration decreases leaving less respiratory carbon available for incorporation into the shell. The decrease in C_m to the shell at larger volumes also coincides with an in increase in the $\delta^{13}C_{Tissue}$ (Fig. 2.2-2.3) for the mussels indicating an increase in the reliance of food resources in the system that are less depleted in $\delta^{13}C$ such as sulfur oxidizing bacteria and phytoplankton. This shift would coincide with an increase in filter feeding in order to support a larger animal volume that is not sufficiently supported through only the nutrition provided by the methanotrophic symbionts.

2.4.5 Temporal Differences in C_m

The Brine Pool site mussels had a steady availability of resources from 1989 to 1991 with no change in C_m for this time period, but in the 2006 sampling a significantly increased C_m indicates an increase in available food for that time period (Fig.2.4). The Bush Hill site has a significant increase in C_m from 1991 to 1992 indicating a quicker increase in the availability of food in a more resource limited setting. These differences on both decadal and yearly time scales demonstrate the large changes in C_m that can occur within cold seep sites and indicate that methane availability can change both quickly (a year) and across decadal time scales.

2.4.6 Between Site Differences in C_m

2.4.6.1 Bush Hill

At the Bush Hill site, there is a smaller range of C_m across volumes and overall mussel volumes do not get as large as the Brine Pool site (Fig.2.2). This indicates widespread food limitation at the site, with mussels limiting the amount of growth that occurs because there is not enough food to support the additional energetic needs that come with a larger volume. This limitation and adverse effect of increased volume is observed in the mussels that range from 4-15 cm^3, where a substantial portion of the C_m values are in the range of 7-10% and fall well below the regression for this site. The site wide limitation in C_m at the Bush Hill site is a sign of a consistent lack of methane availability for the mussel symbionts to process and potentially a corresponding lack of other food available through filter feeding.

2.4.6.2 Brine Pool

Between site comparisons indicate that there is substantially increased C_m in the mussels at the Brine Pool site when compared to the Bush Hill site. This overall site increase in C_m would be driven by increased methane availability at the Brine Pool site relative to the Bush Hill site. This is indicated by the overall averages of C_m at the Brine Pool site being 1.5 times that of the Bush Hill site. This finding is consistent with previous studies that have postulated greater

resource abundance at the Brine Pool through usage of condition indices and growth parameters along with methane concentration measurements at the site (Smith et al. 2000) and poor recent resource availability at the Bush Hill site (Nix et al. 1995).

2.5 Conclusion

Higher C_m values at the Brine Pool site indicate relatively higher food availability and more variability at the site when compared to the Bush Hill site. This is indicative of greater available methane resource abundance for all size classes at the site. There is an ontogenic shift in resource utilization at the Brine Pool site, with small mussels relying on highly localized depleted methane from methanogenesis and larger mussels increasingly relying on a less depleted resource to supplement the nutrition from methanotrophic symbionts. This pattern is not observed at the Bush Hill site and may indicate increased availability of a food source or more filter feeding occurring at the Brine Pool site. C_m is higher at the Brine Pool site consistently for all sampling periods.

The relatively lower C_m observed at the Bush Hill site indicates less food availability at the Bush Hill site across all samplings, but the quick increase in C_m from 1991 to 1992 demonstrates a fairly quick recovery and increased C_m across a small time scale. When this is considered along with the increase across a decadal time scale at the Brine Pool site, it emphasizes the variable nature of methane availability at cold seep sites and the direct effect that it has on *Bathymodiolus childressi*'s metabolism within these systems.

This stable isotope method provides an adequate method to assess relative food availability via a proxy for metabolic activity (C_m) for cold seep mussels between cold seep sites. There is not enough resolution with large population samplings to establish significant within mussel bed differences that have been previously observed for growth rates (Smith et al. 2000), but it appears to be an adequate measurement for relative food availability between sites and across time with a relative minimum of additional cost. With a correction for formalin incorporation, this method is applicable to archived samples in order to gain temporal and spatial perspective on resource availability at sites that have been previously sampled, but not regularly monitored. There is also potential for further development and subsequent application to disarticulated shells with the addition of a proxy for soft tissue that is rarely available after disarticulation in the form of $\delta^{13}C$ of shell protein.

3 Chapter 3: Variance in Soft Tissue Stable Isotope Values Between Sites

3.1 Introduction

Analysis of stable isotope composition has been widely used to model and define food web structures using $\delta^{13}C$ and $\delta^{15}N$, with $\delta^{13}C$ of the individual or population indicating the relative contribution of each known dietary source. $\delta^{15}N$ is an indicator of relative trophic level (Peterson and Fry 1987). Adjustment to measured isotope values are necessary to compensate for trophic level effects (Newsome et al. 2007). Sulfur isotopes have been used to indicate the level of benthic interaction in food resources for predators (Peterson 1999). Variations in stable isotope values among individuals within a population have been suggested as a measure of niche breadth (Barnes et al. 2008; Bearhop et al. 2004). The degree of variation in individual isotope values may be an important indicator of variation when resources can only be implied and have not been measured.

Cold seep sites are uniquely heterogeneous, reducing ecosystems with widespread availability of CNS sources such as methane, ammonium, and hydrogen sulfide not commonly seen utilized by biota in other systems (Cordes et al. 2010c; Kennicutt et al. 1992). In these systems, symbiont-bearing organisms make use of these basal chemical resources as a large portion of their diets via microbially-mediated transformations. This study focuses on one such symbiont bearing species, the mussel *Bathymodiolus childressi*, with the goal of delineating the stable isotope variation within these sites using the CNS isotope values from a large sampling of individuals. *B. childressi* houses a methanotrophic symbiont and is thought to rely extensively on the available methane resources that are in their proximity in the environment. However, the animal retains a functional gut and the capability of filter feeding (Page et al. 1990), but the extent of reliance on filter feeding has not been studied in detail.

The resources and fractionations associated with the chemical pathways present in cold seeps are known to some extent from previous studies, as summarized in Table 3.1. Current isotope values include values for phytoplankton (Peterson and Fry 1987; Wells and Rooker 2009), biogenic (Sassen et al. 1999) and thermogenic methane (Sassen et al. 2004), the fractionation associated with the oxidation of methane by a Type 1 methanotroph (Templeton et al. 2006), sulfur oxidizing bacteria (SOB) (Demopoulos et al. 2010), seawater sulfate (Rees et al. 1978), bottom water nitrate (Liu and Kaplan 1989), the fractionation that results from ammonium uptake (Hoch et al. 1992), hydrogen sulfide (Fu 1998), and a range for the fractionation of the oxidation of hydrogen sulfide (Canfield 2001). The degree of variation in these resources is not well known, and the goal of this study is to utilize the variation in the stable isotope values of mussel populations in order to better constrain the variation in basal resource values.

Table 3.1: Known Literature Values for Sources in Cold Seep Environments. ε values are fractionation values from environmental substrate into organism tissue or biologically mediated chemical transformation with (–) indicating a depletion from substrate value into tissue or chemical transformation into product and (+) indicating an enrichment from substrate value into tissue or chemical transformation into product.

Sources (all values in ‰)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	δD	ε	**References**
Methane Brine Pool	-64			-165, -200		Sassen (1999)
Methane Bush Hill	-45.4			-178		Sassen (2004)
$CH_4 \rightarrow \delta^{13}C_{tissue}$ Type 1 methanotroph[a]					-(15-25)	Templeton (2006)
POM Gulf of Mexico (surface)	-21.5	2.8				Wells and Rooker (2009)
Plankton Estuarine	-20		21			Peterson and Fry (1987)
Bottom Water Nitrate		5-7				Liu and Kaplan 1989
$\delta^{15}N$ Trophic Enrichment					3.4	Vander Zanden et al. (2001)
Ammonium uptake $NH4^{+} \rightarrow \delta^{15}N_{tissue}$					-(14-27)	Hoch (1992)
Sulfur Oxidizing Bacteria	-31	-2.5	very depleted			Demopolous (2010)
Seawater Sulfate			21			Rees (1978)
Hydrogen Sulfide (sediment)[b]			12.1			Fu (1998)
Sulfate Reduction to Hydrogen Sulfide					-9	Fu (1998)
Hydrogen Sulfide Oxidation to Sulfate					-(32-58)	Canfield and Thamdrup (1994)

a- Pure cullture *M. methinica* at 22-24°C
b-Bush Hill 2cm depth

3.2 Methods

Samples were processed and analyzed in the same manner as the tissue samples in Chapter 2 and were corrected for the use of formalin as a fixative in the same manner described before using a 15% incorporation rate and a $\delta^{13}C$ value of -41.7‰ for formalin.

3.3 Results

3.3.1 Data Analysis

Overall sample size in this study is 257 individuals. For all sites and within bed samplings, average and standard deviation were calculated for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, mass of nitrogen, mass of carbon, and mass of sulfur. Means of %N, %C, and %S are also reported. These are reported in tables for each sampling design. The Brine Pool (n=134) site (Table 3.4 A and B) was sampled sufficiently to allow for the analysis of variation between years (1989 n=61, 1991 n=27, and 2006 n=46, Table 3.2 A and B), spatially (Interior n=55 and Edge n=33, North n= 72 and South n=62, Table 3.3A and B) and between sites. The Bush Hill (n=123) site was sampled sufficiently to allow for the analysis of variation within CNS isotopes between years (1991 n=67 and 1992 n=56, Table 3.2 A and B) and within the site (Interior n=63 and Edge n=60, Bed1 n=71, Bed 2 n=31, and Bed 1A n=21, Table 3.3 A and B).

3.4 Discussion

The variability in the stable isotope composition of *Bathymodiolus childressi* is an indicator of the range of resources available to the population at each site. The variation found in

tissue is caused by multiple factors. These can include differences in resources being used at both sites. Also, changes may occur along geochemical pathways and associated fractionations of the resource before it reaches the organism. Finally, there are physiological differences between animals that affect the extent of fractionation during resource utilization. As a conglomeration of variability caused by several individual differences, the differences in variability can indicate the relative stability of the sources of each tracer component. This allows for the interpretation of the relative variability of each tracer across time, spatially, and between sites.

3.4.1 Brine Pool

3.4.1.1 Temporal Effects on Standard Deviation

The Brine Pool site showed a large difference in standard deviations for $\delta^{15}N$ across sampling years, this large difference in variability is partially due to a unique sampling group of 5 individuals for Brine Pool 1991 North Edge that were ~18‰ more enriched for $\delta^{15}N$ and ~10‰ more depleted for $\delta^{34}S$ (Table 3.2 A). Due to these outliers and the large source of variability for a single sampling year, the Brine Pool is reported as both with BP 1991 North Edge and without for analyses. The existence of the small outlier group of individuals apparently utilizing a separate suite of resources highlights the difficulty in accounting for all resources in ecological samplings. Without the BP 1991 North Edge outlier group included, the differences in standard deviation between the sampling years is most likely to reflect the isotopic variability of the resources being utilized by the more representative portion of the mussels for that site. Of the three sampled isotopes, $\delta^{15}N$ has the lowest standard deviation across sampling years, $\delta^{13}C$ has the highest standard deviation across sampling years, with the variability of $\delta^{34}S$ falling between the two extremes (Table 3.2 A). Elevated variability in $\delta^{13}C$ is expected when multiple distinct carbon sources in the sense of methane versus detritus or microbes are being utilized. Alternatively, it may reflect the variability in $\delta^{13}C$ of the methane source or variability in the concentration of the methane available at the site. Differences in concentration will affect the amount of fractionation (ε= -(15-25)‰ for $\delta^{13}C$ Table 3.1) that occurs as methane is incorporated into the tissue. The smaller standard deviations in $\delta^{15}N$ between years and relative homogeneity in standard deviation across sampling years in the mass of nitrogen is indicative of a relatively stable source of ammonium within the Brine Pool resulting in extremely depleted $\delta^{15}N$ values (-14‰). Given the large fractionation associated with ammonium incorporation into tissue (ε=-(14-27)‰ Table 3.1), a relatively low maximum standard deviation of 1.8‰ across all years sampled can be interpreted as a relatively stable amount of ammonium being incorporated in to the tissue of *Bathymodiolus childressi* at this site across all years sampled. The standard deviations for $\delta^{34}S$ is fairly high, indicating variable amounts of incorporation of a uniquely depleted food source (possibly sulfur oxidizing bacteria) or variable amounts of exposure to hydrogen sulfide throughout the site.

Table 3.2: Temporal Effects for Both Sites. A) Means and standard deviations for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ for both sites. The first 1991 Brine Pool sampling includes the significant outlier group, the second is with the outliers removed. B) Means and standard deviations for mass of nitrogen, carbon, and sulfur, %N, %C, and %S. The 1991 outlier group is not significantly different for these parameters, so they are not reported with them removed.

A.

Sampling Year	Site	n	$\delta^{15}N$ (‰)	Standard Deviation	$\delta^{13}C$ (‰)	Standard Deviation	$\delta^{34}S$ (‰)	Standard Deviation
1989	Brine Pool	61	-14.9	1.8	-63.5	5.4	9.5	3.0
1991	Brine Pool	27	-11.4	7.5	-60.4	2.4	9.5	4.4
1991	Brine Pool	22	-14.9	1.0	-61.0	2.8	11.6	0.7
2006	Brine Pool	46	-16.1	1.0	-62.9	4.5	10.8	1.2
1991	Bush Hill	67	4.8	1.0	-36.7	1.0	-1.0	3.3
1992	Bush Hill	56	3.7	1.1	-39.8	1.7	2.9	3.2

B.

Sampling Year	Site	n	N (mg)	Standard Deviation	C (mg)	Standard Deviation	S (mg)	Standard Deviation	%N	%C	%S
1989	Brine Pool	61	723	85	2854	301	82	10	12	48	1.4
1991	Brine Pool	27	761	97	2940	319	74	10	13	49	1.2
2006	Brine Pool	46	773	103	2850	387	92	17	13	48	1.5
1991	Bush Hill	67	780	83	2820	298	79	11	13	48	1.3
1992	Bush Hill	56	745	90	2800	311	72	10	12	47	1.2

3.4.1.2 Spatial Effects

3.4.1.2.1 Interior and Edge Effects on Standard Deviation

Differences within mussel beds at the Brine Pool site showed significant differences in standard deviations for both Interior and Edge samplings. The Interior sampling has a higher standard deviation of 6.2‰ for $\delta^{13}C$, while both δ15N 1.3‰ and δ34S 1.6‰ were lower (Table 3.3 A). The Edge sampling excluding BP 1991 North Edge outliers had a lower standard deviation for $\delta^{13}C$ of 2.9‰, a similar standard deviation for $\delta^{15}N$ of 1.6, and an increased standard deviation for $\delta^{34}S$ of 3.2‰. The uniform standard deviation between Interior to Edge for $\delta^{15}N$ after excluding BP 1991 North Edge outliers indicate a relatively uniform availability of ammonium across the Brine Pool site. If ammonium varies in spatial availability or temporally, it would be expected that the fractionation would be more variable and thereby show greater variability across the mussel bed. The decrease in $\delta^{13}C$ variability from Interior to Edge could be due to either difference in fractionation as concentrations methane become more limiting towards the edge of the mussel bed or represent a shift in the availability of different food to the mussels. The greater variability of $\delta^{34}S$ in the Edge samplings indicates either an increase in the utilization

of a depleted food source or an increase in the amount of available hydrogen sulfide in portions of the edge of the site.

Table 3.3: Spatial Effects for Both Sites. A) Means and standard deviations for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ for both sites. The first samplings for both Brine Pool Edge and Brine Pool North include the 1991 Brine Pool North Edge outlier group, the second is with those outliers removed. B) Means and standard deviations for mass of nitrogen, carbon, and sulfur, %N, %C, and %S. The outlier group is not significantly different for these parameters, so they are not reported with them removed.

A.

Within Bed	Site	n	$\delta^{15}N$ (‰)	Standard Deviation	$\delta^{13}C$ (‰)	Standard Deviation	$\delta^{34}S$ (‰)	Standard Deviation
Interior	Brine Pool	55	-15.4	1.3	-64.4	6.2	11.2	1.6
Edge	Brine Pool	33	-11.0	6.6	-59.6	2.4	6.6	4.0
Edge	Brine Pool	28	-13.7	1.6	-59.9	2.9	7.7	3.2
North	Brine Pool	72	-13.4	5.1	-61.9	6.5	9.0	3.4
North	Brine Pool	67	-14.7	1.8	-62.2	6.6	9.6	2.6
South	Brine Pool	62	-15.9	1.0	-63.6	3.2	11.1	1.7
Interior	Bush Hill	63	4.4	1.4	-38.3	2.8	0.6	4.2
Edge	Bush Hill	60	4.2	0.9	-38.1	1.5	1.0	3.4
Bed 1	Bush Hill	71	4.8	0.9	-37.4	1.5	-1.0	1.5
Bed 1A	Bush Hill	31	4.4	1.2	-37.4	1.3	0.8	3.7
Bed 2	Bush Hill	21	2.9	1.0	-41.7	2.0	6.9	0.9

B.

Within Bed	Site	n	N (mg)	Standard Deviation	C (mg)	Standard Deviation	S (mg)	Standard Deviation	%N	%C	%S
Interior	Brine Pool	55	743	96	2910	320	78	8.9	12.5	48.9	1.3
Edge	Brine Pool	23	721	78	2832	284	83	12.5	12.2	47.9	1.4
North	Brine Pool	72	743	91	2891	300	83	15.4	12.4	48.1	1.4
South	Brine Pool	52	753	102	2846	374	85	12.4	12.8	48.4	1.4
Interior	Bush Hill	63	771	85	2787	316	75	10.3	13.2	47.6	1.3
Edge	Bush Hill	50	756	91	2836	290	77	11.4	12.6	47.1	1.3
Bed 1	Bush Hill	71	760	88	2794	288	75	10.1	12.9	47.4	1.3
Bed 1A	Bush Hill	31	763	86	2825	362	74	10.3	12.8	47.3	1.2
Bed 2	Bush Hill	21	735	80	2748	244	74	11.0	12.5	46.9	1.3

3.4.1.2.2 North and South Effects on Standard Deviation

Differences within mussel beds at the Brine Pool site showed significant differences in variability between the North and South sides of the site. The North sampling excluding BP 1991 North Edge outliers had higher standard deviations with $\delta^{13}C$ being the largest with 6.6‰, and $\delta^{34}S$ with 2.6‰ and $\delta^{15}N$ with 1.8‰ being smaller (Table 3.3 A). The standard deviations associated with the South site are much smaller for all isotope values. This indicates more stability of available resources for the South side of the Brine Pool when compared to the North side with less reliance on alternative food sources and less variability in the ammonium being provided to that side of the site. This decrease in variability is also supported by the geological

structure at the site where the Brine overflows to the South, flowing beneath the mussels as it leaves the central pool (R. S. Carney, personal communication).

3.4.2 Bush Hill

3.4.2.1 Temporal

The standard deviations associated with all parameters measured are relatively homogenous at the Bush Hill site for the 1991 to 1992 samplings. The shift in standard deviation for $\delta^{13}C$ from 1991 of 1‰ to 1.7‰ in 1992 likely indicates a shift in resource utilization by the mussels at the site or differences in the fractionation associated with methane indicating a change in availability (Table 3.2 A). The standard deviations in the other measured stable isotopes changed very little indicating relative stability in variability for both $\delta^{15}N$ and $\delta^{34}S$, which had standard deviations of around 1‰ and 3.3‰ respectively. The large SD associated with $\delta^{34}S$ is indicative of a large range of incorporation of depleted sulfur resulting in the average for $\delta^{34}S$ shifting from 1991 (-1‰) to 1992 (2.9‰). This large shift in $\delta^{34}S$ average without a corresponding increase in standard deviation likely indicates increased availability of non-depleted sulfur sources within the site in 1992.

3.4.2.2 Spatial

3.4.2.2.1 Interior and Edge Effects on Standard Deviation

Differences between the within mussel bed Interior and Edge samplings at Bush Hill showed little difference in means for all isotopes but considerable differences in standard deviation for both $\delta^{13}C$ and $\delta^{34}S$. The standard deviation for $\delta^{13}C$ decreased from 2.8‰ to 1.5‰ indicating less variability in the carbon values for the population (Table 3.3 A). This change indicates either a change in the fractionation associated with methane uptake or a difference in the amount of utilization of resources in the site. The standard deviation for $\delta^{34}S$ decreased from 4.2‰ to 3.2‰ indicating less variability in the interior than in the edge of the mussel bed. This most likely indicates a shift in resource utilization in 1992.

3.4.2.2.2 Bed Effects on Standard Deviation

Differences between bed sampling at Bush Hill showed little differences for the means or standard deviations for $\delta^{15}N$. The largest standard deviation was for $\delta^{34}S$ for Bed 1A of 3.7, which was higher than those for Bed 1 or Bed 2 (Table 3.3 A). This change also coincides with a large shift in the range of averages $\delta^{34}S$ from these beds from -1.0‰ to 6.9‰. This indicates a larger amount of variability for the Bed 1A specimens and is likely a result of a wide range of utilization of depleted sulfur sources between the mussel beds. The average for $\delta^{13}C$ shifts between -37.4‰ for Bed 1 and Bed 1A to -41.7‰ for Bed 2 indicating increased reliance on methane for mussels at Bed 2.

3.4.3 Between Site Effects

The variability associated with stable isotope values found at the Brine Pool and at Bush hill is considerably different. The Brine Pool has much higher standard deviations for both $\delta^{15}N$

of 4‰ and $\delta^{13}C$ of 5.3‰ versus 1‰ and 1.5‰ and similar standard deviations associated with $\delta^{34}S$ of 3‰ for each site (Table 3.4 A). These findings are reasonable when the differences in available resources and the fractionations that are associated with them are considered. The large amounts of ammonium utilization at the Brine Pool introduce a large source of variability not encountered at Bush Hill. There is also considerably more variation in $\delta^{13}C$ observed as the range of $\delta^{13}C$ of sources for the Brine Pool is considerably extended due to the availability of biogenic methane at the site with a $\delta^{13}C$ value of -64‰ versus the thermogenic methane that is available at the Bush Hill site with a $\delta^{13}C$ value of -45.4‰ (Table 3.1). The differences in resource stable isotope values are reflected in the extreme difference in means between the two sites. The Brine Pool has a $\delta^{15}N$ value of -14.6‰ and a $\delta^{13}C$ value of -62.7‰ which are indicative of the utilization of ammonium and biogenic methane. Bush Hill has values of 4.6‰ and -37.5‰ respectively, indicative of little utilization of ammonium and the presence of thermogenic methane. These extreme differences in resource values between sites are a strong argument for the development of independent mixing models using at least two unique resources for each site for further analysis of mussel resource utilization. The similarity in standard deviations between the sites in $\delta^{34}S$ indicates the similarity in resources for sulfur, but the large difference in means for $\delta^{34}S$ between the Brine Pool with 10‰ and Bush Hill with -0.3‰ indicate a higher amount of utilization at the Bush Hill site of a resource with highly depleted sulfur.

Table 3.4: Between Site Effects. A) Means and standard deviations for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ for both sites. B) Means and standard deviations for mass of nitrogen, carbon, and sulfur, %N, %C, and %S for both sites.

A.

Site	n	δ15N (‰)	Standard Deviation	δ13C (‰)	Standard Deviation	δ34S (‰)	Standard Deviation
Brine Pool	134	-14.6	4.0	-62.7	5.3	10.0	3.0
Bush Hill	123	4.6	1.0	-37.5	1.5	-0.3	3.0

B.

Site	n	N (mg)	Standard Deviation	C (mg)	Standard Deviation	S (mg)	Standard Deviation	%N	%C	%S
Brine Pool	134	748	96	2870	336	84	14	13	48	1.4
Bush Hill	123	766	87	2810	297	76	11	13	47	1.3

3.5 Conclusion

The marked contrasts in means and ranges of stable isotope values between these two cold seeps indicate the utilization of unique resources available at each site. The distinct differences in site-specific resource suites are very obvious in the differences in ammonium

utilization and the differences in methane types between the sites. The exception to site-specific differences is sulfur, which exhibited no difference in standard deviation between sites, but substantially different $\delta^{34}S$ means. This indicates similar availability of resources between sites for sulfur, but different rates of utilization of those resources. These findings argue strongly for the necessity of a mixing model in order to compare resource utilization by the populations at different sites. The extreme heterogeneity of resources available between sites hampers simple interpretation of the stable isotope data. This is further emphasized by the appearance of a small outlier subset of individuals (BP 1991 North Edge) that are reliant upon a unique set of resources which indicate extreme heterogeneity in resources. The change in resource availability arose quickly and was heavily utilized by a small subset of the population that was subsequently not observed during further sampling. Future work should involve both the measurement of basal resources and the utilization of separate mixing models for interpretation of stable isotope data for mussel beds associated with separate cold seeps.

Conclusion

Addressing the questions posed as objectives in Chapter 1.

1. Do the means and variances of stable isotopes in mussel soft tissues indicate steady state or changing conditions at the two sites examined?

The means and variance of stable isotopes found in this study indicate a large degree of heterogeneity between the Bush Hill and Brine Pool cold seep sites. The changes in resource availability and resource utilization by the mussels at each seep strongly indicate that the steady state hypothesis for these cold seeps is not correct and that they exhibit changing conditions across both temporal and spatial scales. These findings support the further use of unique mixing models for resource utilization at each site and strongly emphasize the need for further sampling basal resources available at each cold seep individually.

2. Does the carbon isotope composition of mussel shells provide similar information about populations which might be used when tissue is not available?

The carbon isotope composition of mussel shells is a useful indicator of relative food availability between sites. This method does not provide enough resolution for easy analysis of within site changes without large sampling sizes and available soft tissue. The utilization of shell protein as a proxy for $\delta^{13}C_{tissue}$ is possible and would provide a variable for this mixing model that is routinely unknown for disarticulated shells. Without this proxy, analysis of shells without associated soft tissue would be highly speculative as the ranges of $\delta^{13}C_{Shell}$ overlap between the sites in this study with clearly different biogenic and thermogenic methane resources.

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Appendix

A.1 Brine Pool 1989 and 1991 Shell Analysis Data

Table A.1: All Sample data for the Brine Pool 1989 and 1991 Samplings for Chapter 2.

Sample ID	Site	Bed	Position	Year	Half Shell Volume (cm^3)	$\delta^{13}C$ Shell (‰)	$\delta^{13}C$ Tissue (‰)	$\%C_m$
2598.7.1(2).2	Brine Pool	South	Edge	1989	62.2	-11.3	-60.6	17.5
2598.7.1(2).3	Brine Pool	South	Edge	1989	68.8	-11.3	-62.1	17.0
2598.8.1.5	Brine Pool	South	Edge	1989	4.8	-6.8	-67.8	12.2
2598.8.1.6	Brine Pool	South	Edge	1989	5.1	-6.5	-68.8	11.5
2598.8.1.07f	Brine Pool	South	Edge	1989	5.1	-6.4	-67.2	11.7
2598.8.1.9	Brine Pool	South	Edge	1989	4.6	-4.2	-72.3	7.9
3145.6.1.07F	Brine Pool	North	Interior	1991	59.0	-8.2	-60.3	12.6
3145.6.1.05F	Brine Pool	North	Interior	1991	104.5	-7.2	-60.9	10.2
3145.6.1.08F	Brine Pool	North	Interior	1991	37.0	-7.6	-60.7	12.3
3145.6.1.10F	Brine Pool	North	Interior	1991	5.4	-8.2	-59.6	15.9
3145.6.1.06F	Brine Pool	North	Interior	1991	88.6	-8.5	-59.4	12.9
3145.6.1.03F	Brine Pool	North	Interior	1991	23.2	-9.6	-60.9	16.1
3145.6.1.11F	Brine Pool	North	Interior	1991	58.0	-7.4	-61.3	11.1
3145.6.1.1F	Brine Pool	North	Interior	1991	7.2	-7.0	-51.3	15.9
2598.9.1(2).06F	Brine Pool	North	Interior	1989	3.5	-9.2	-77.8	13.9
2598.10.1.01F	Brine Pool	North	Interior	1989	53.9	-7.6	-61.8	11.5
2598.10.1.04f	Brine Pool	North	Interior	1989	3.7	-5.0	-65.1	10.3
2598.2.1.5	Brine Pool	South	Interior	1989	28.6	-5.9	-56.7	10.4
2598.4.1.5	Brine Pool	South	Interior	1989	10.8	-6.1	-62.3	11.1
2598.5.1(2).4	Brine Pool	South	Interior	1989	5.2	-4.5	-62.1	9.6
2598.5.1.1	Brine Pool	South	Interior	1989	51.9	-8.6	-62.2	13.0
2598.5.1.2	Brine Pool	South	Interior	1989	41.1	-8.3	-60.8	13.2
3145.5.1.01F	Brine Pool	North	Edge	1991	11.8	-7.6	-57.7	14.4
3145.5.1.02F	Brine Pool	North	Edge	1991	15.2	-8.1	-56.7	15.1
3145.5.1.03F	Brine Pool	North	Edge	1991	14.1	-8.9	-59.3	16.0
3145.5.1.05F	Brine Pool	North	Edge	1991	22.7	-6.6	-56.8	11.9
2598.11.1(2).03F	Brine Pool	North	Edge	1989	10.0	-8.6	-59.1	16.0
2598.11.1.05F	Brine Pool	North	Edge	1989	2.9	-4.4	-61.3	10.2
2598.12.1(2).03F	Brine Pool	North	Edge	1989	5.5	-5.2	-61.3	10.6
2598.11.1.1	Brine Pool	North	Edge	1989	21.9	-7.7	-59.2	13.4
2598.11.1.4	Brine Pool	North	Edge	1989	40.9	-9.3	-59.8	15.0
2598.11.1.6	Brine Pool	North	Edge	1989	4.4	-4.9	-63.3	10.2
2598.12.1(2).1	Brine Pool	North	Edge	1989	5.5	-6.8	-57.7	14.0
2598.12.1.2	Brine Pool	North	Edge	1989	33.1	-7.2	-58.4	12.1
2598.12.1.3	Brine Pool	North	Edge	1989	71.7	-7.6	-57.4	11.9
2598.5.1(2).05F	Brine Pool	South	Interior	1989	6.8	-5.5	-63.1	10.7
3145.3.1.04F	Brine Pool	South	Interior	1991	41.7	-9.7	-61.9	15.1
3145.3.1.05F	Brine Pool	South	Interior	1991	17.3	-10.0	-65.0	15.9
3145.3.1.06F	Brine Pool	South	Interior	1991	4.7	-4.8	-65.2	9.6
3145.3.1.08F	Brine Pool	South	Interior	1991	6.1	-5.5	-63.6	10.6
2598.9.1(2).1	Brine Pool	North	Interior	1989	10.0	-11.9	-74.6	17.1
2598.9.1(2).2	Brine Pool	North	Interior	1989	66.1	-13.7	-69.2	18.7
2598.9.1(2).3	Brine Pool	North	Interior	1989	7.0	-11.0	-69.0	17.6
2598.9.1(2).5	Brine Pool	North	Interior	1989	16.4	-10.5	-70.7	15.5
2598.9.1(2).9	Brine Pool	North	Interior	1989	3.0	-8.2	-76.1	13.2
2598.9.1.2	Brine Pool	North	Interior	1989	14.8	-12.0	-73.1	17.1
2598.9.1.3	Brine Pool	North	Interior	1989	2.7	-9.1	-80.9	13.5

A.2 Brine Pool 2006 Shell Analysis Data

Table A.2: All Sample data for the Brine Pool 2006 sampling for Chapter 2.

Sample ID	Site	Bed	Position	Year	Half Shell Volume (cm^3)	$\delta^{13}C$ Shell (‰)	$\delta^{13}C$ Tissue (‰)	$\%C_m$
3522.02F	Brine Pool	South		2006	8.5	-10.2	-64.1	17.4
3522.03F	Brine Pool	South		2006	1.4	-5.4	-68.6	11.4
3522.05F	Brine Pool	South		2006	11.9	-11.9	-65.9	19.1
3522.08f	Brine Pool	South		2006	8.2	-10.0	-65.2	16.9
3522.09f	Brine Pool	South		2006	10.8	-10.6	-62.7	18.1
3522.11f	Brine Pool	South		2006	33.6	-11.1	-63.2	17.4
3522.12f	Brine Pool	South		2006	20.5	-13.0	-64.6	20.6
3522.13F	Brine Pool	South		2006	1.7	-7.9	-66.0	15.4
3522.18F	Brine Pool	South		2006	23.0	-13.8	-61.1	22.8
3522.19f	Brine Pool	South		2006	14.1	-11.0	-63.1	18.3
3522.20F	Brine Pool	South		2006	11.1	-10.5	-66.5	16.9
3522.21F	Brine Pool	South		2006	7.3	-9.9	-69.2	15.8
3522.23f	Brine Pool	South		2006	11.1	-9.1	-61.6	16.0
3522.25F	Brine Pool	South		2006	16.6	-12.6	-65.1	20.0
3522.30f	Brine Pool	South		2006	5.3	-13.4	-67.3	21.8
3522.34F	Brine Pool	South		2006	3.3	-8.8	-62.3	16.8
3522.35f	Brine Pool	South		2006	5.2	-6.3	-57.5	13.3
3522.36F	Brine Pool	South		2006	6.1	-8.9	-64.3	15.7
3522.39F	Brine Pool	South		2006	5.6	-11.0	-69.4	17.7
3522.45F	Brine Pool	South		2006	1.9	-6.8	-68.1	13.2
3522.46F	Brine Pool	South		2006	2.0	-5.1	-69.3	10.5
3522.48F	Brine Pool	South		2006	13.8	-9.3	-60.2	16.5
3522.51f	Brine Pool	South		2006	11.2	-13.1	-66.4	20.8
3522.52F	Brine Pool	South		2006	57.1	-12.4	-59.0	20.0
3522.54f	Brine Pool	South		2006	6.6	-9.6	-63.3	17.1
3522.55F	Brine Pool	South		2006	18.1	-13.1	-63.9	21.0
3522.60F	Brine Pool	South		2006	30.7	-12.0	-62.7	19.0
3522.61F	Brine Pool	South		2006	6.5	-10.6	-67.0	17.6
3522.64F	Brine Pool	South		2006	39.6	-15.2	-68.0	21.9
3522.65F	Brine Pool	South		2006	46.5	-11.9	-61.8	18.6
3522.70F	Brine Pool	South		2006	21.3	-12.2	-65.4	18.9
3522.71F	Brine Pool	South		2006	2.9	-7.1	-70.7	12.7
3522.76F	Brine Pool	South		2006	18.8	-10.1	-62.2	16.8
3522.77F	Brine Pool	South		2006	1.6	-8.0	-70.7	14.6
3521.02f	Brine Pool	North		2006	89.8	-9.8	-56.8	15.5
3521.03f	Brine Pool	North		2006	89.4	-8.4	-56.2	13.4
3521.04f	Brine Pool	North		2006	70.8	-8.9	-56.9	14.4
3521.05f	Brine Pool	North		2006	89.2	-9.9	-57.6	15.6
3521.06f	Brine Pool	North		2006	50.0	-8.5	-56.8	14.2
3521.07f	Brine Pool	North		2006	81.9	-9.2	-57.9	14.4
3521.08f	Brine Pool	North		2006	96.8	-11.0	-58.0	17.2
3521.09f	Brine Pool	North		2006	43.7	-9.5	-57.5	15.9
3521.10F	Brine Pool	North		2006	77.8	-9.6	-54.3	16.1
3521.13F	Brine Pool	North		2006	74.7	-8.6	-55.2	14.1
3521.14F	Brine Pool	North		2006	122.5	-8.9	-57.0	13.6

A.3 Bush Hill 1991 Shell Analysis Data

Table A.3: All Sample data for the1991 Bush Hill Samplings for Chapter 2.

Sample ID	Site	Bed	Position	Year	Half Shell Volume (cm^3)	$\delta^{13}C$ Shell (‰)	$\delta^{13}C$ Tissue (‰)	$\%C_m$
3139.2.1.01F	Bush Hill	Bed 1	Interior	1991	7.8	-1.5	-35.8	7.3
3139.2.1.02F	Bush Hill	Bed 1	Interior	1991	9.6	-2.2	-35.8	8.8
3139.2.1.09F	Bush Hill	Bed 1	Interior	1991	4.3	-2.5	-35.8	11.4
3139.2.1.14F	Bush Hill	Bed 1	Interior	1991	10.8	-2.7	-36.8	9.6
3139.3.1.03f	Bush Hill	Bed 1	Interior	1991	3.6	-1.9	-36.0	10.0
3139.3.1.08F	Bush Hill	Bed 1	Interior	1991	1.6	-0.7	-36.3	8.4
3139.3.1.12F	Bush Hill	Bed 1	Interior	1991	3.4	-1.6	-35.9	9.3
3139.3.1.15F	Bush Hill	Bed 1	Interior	1991	5.7	-2.0	-36.2	9.3
3139.3.1.20F	Bush Hill	Bed 1	Interior	1991	10.7	-2.0	-36.9	7.8
3139.3.1.21F	Bush Hill	Bed 1	Interior	1991	9.7	-1.5	-36.0	6.8
3139.1.1.01F	Bush Hill	Bed 1	Edge	1991	10.7	-1.5	-35.7	6.6
3139.1.1.02F	Bush Hill	Bed 1	Edge	1991	8.0	-2.0	-36.7	8.3
3139.1.1.08F	Bush Hill	Bed 1	Edge	1991	6.7	-1.1	-36.5	6.3
3139.1.1.10F	Bush Hill	Bed 1	Edge	1991	1.6	-0.2	-36.2	7.0
3139.1.1.13F	Bush Hill	Bed 1	Edge	1991	14.3	-2.0	-36.6	7.1
3139.4.1.02F	Bush Hill	Bed 2	Interior	1991	3.6	-1.6	-36.3	9.2
3139.4.1.03F	Bush Hill	Bed 2	Interior	1991	1.4	-0.1	-36.2	7.2
3139.4.1.06F	Bush Hill	Bed 2	Interior	1991	2.2	-0.7	-36.7	7.8
3139.4.1.13F	Bush Hill	Bed 2	Interior	1991	2.8	-1.3	-36.2	8.8
3139.4.1.16F	Bush Hill	Bed 2	Interior	1991	4.0	-2.8	-36.3	12.1
3139.4.1.17F	Bush Hill	Bed 2	Interior	1991	4.1	-1.8	-35.9	9.6
3139.5.1.03F	Bush Hill	Bed 2	Interior	1991	14.7	-2.9	-37.2	9.4
3139.5.1.09F	Bush Hill	Bed 2	Interior	1991	9.9	-3.4	-37.4	11.5
3139.7.1.01F	Bush Hill	Bed 2	Edge	1991	5.6	-1.7	-38.7	8.0
3139.7.1.03F	Bush Hill	Bed 2	Edge	1991	1.7	-0.4	-36.9	7.3
3139.7.1.04F	Bush Hill	Bed 2	Edge	1991	18.9	-3.1	-37.3	9.3
3139.7.1.07F	Bush Hill	Bed 2	Edge	1991	9.7	-2.9	-38.1	10.1
3139.7.1.08F	Bush Hill	Bed 2	Edge	1991	5.6	-2.0	-38.0	8.8
3139.7.1.10F	Bush Hill	Bed 2	Edge	1991	24.2	-4.7	-37.8	12.7
3139.7.1.11F	Bush Hill	Bed 2	Edge	1991	14.2	-3.4	-38.2	10.4
3139.7.1.12F	Bush Hill	Bed 2	Edge	1991	17.3	-4.1	-37.5	12.1
3139.7.1.15F	Bush Hill	Bed 2	Edge	1991	17.3	-4.2	-37.4	12.4

A.4 Bush Hill 1992 Shell Analysis Data

Table A.4: All Sample data for the1992 Bush Hill Samplings for Chapter 2.

Sample ID	Site	Bed	Position	Year	Half Shell Volume (cm^3)	$\delta^{13}C$ Shell (‰)	$\delta^{13}C$ Tissue (‰)	$\%C_m$
3269.11.1.01F	Bush Hill	Bed 1	Interior	1992	12.0	-3.2	-38.3	10.3
3269.11.1.02F	Bush Hill	Bed 1	Interior	1992	10.4	-4.7	-37.1	14.9
3269.11.1.03F	Bush Hill	Bed 1	Interior	1992	12.5	-3.4	-39.1	10.5
3269.12.1.01F	Bush Hill	Bed 1	Interior	1992	10.2	-3.1	-37.1	10.7
3269.12.1.02F	Bush Hill	Bed 1	Interior	1992	9.3	-3.8	-37.9	12.4
3274.2.1.02F	Bush Hill	Bed 1A	Interior	1992	1.6	-1.7	-43.5	9.2
3274.2.1.04F	Bush Hill	Bed 1A	Interior	1992	1.3	-2.0	-43.5	10.3
3274.2.1.09F	Bush Hill	Bed 1A	Interior	1992	3.8	-2.5	-44.0	9.4
3274.2.1.10F	Bush Hill	Bed 1A	Interior	1992	4.3	-2.3	-40.1	9.5
3274.2.1.11F	Bush Hill	Bed 1A	Interior	1992	1.1	-1.2	-42.9	8.9
3274.2.1.13F	Bush Hill	Bed 1A	Interior	1992	2.1	-1.6	-47.0	7.8
3274.3.1(2).03F	Bush Hill	Bed 1A	Interior	1992	4.5	-2.5	-42.5	9.5
3274.3.1(2).05F	Bush Hill	Bed 1A	Interior	1992	2.7	-1.8	-41.0	9.0
3274.3.1(2).06F	Bush Hill	Bed 1A	Interior	1992	10.7	-4.1	-41.0	11.9
3274.3.1(2).08F	Bush Hill	Bed 1A	Interior	1992	7.6	-2.5	-41.8	8.5
3274.3.1(2).12F	Bush Hill	Bed 1A	Interior	1992	12.8	-3.3	-41.4	9.6
3274.3.1(2).15F	Bush Hill	Bed 1A	Interior	1992	6.1	-3.5	-42.1	11.3
3274.3.1.02F	Bush Hill	Bed 1A	Interior	1992	4.2	-1.5	-43.9	6.9
3274.3.1.17F	Bush Hill	Bed 1A	Interior	1992	35.9	-4.2	-40.7	9.9
3274.3.1.20F	Bush Hill	Bed 1A	Interior	1992	2.1	-1.4	-41.6	8.5
3274.1.1.01F	Bush Hill	Bed 1A	Edge	1992	11.0	-4.3	-39.9	12.7
3274.1.1.04F	Bush Hill	Bed 1A	Edge	1992	3.5	-2.0	-41.3	9.0
3274.1.1.06F	Bush Hill	Bed 1A	Edge	1992	2.8	-1.5	-41.5	8.2
3274.1.1.08F	Bush Hill	Bed 1A	Edge	1992	2.2	-1.4	-39.7	8.9
3274.1.1.11F	Bush Hill	Bed 1A	Edge	1992	11.9	-2.6	-39.6	8.5
3274.2.1.02F	Bush Hill	Bed 1A	Interior	1992	1.6	-1.7	-43.5	9.2
3274.2.1.04F	Bush Hill	Bed 1A	Interior	1992	1.3	-2.0	-43.5	10.3
3274.2.1.09F	Bush Hill	Bed 1A	Interior	1992	3.8	-2.5	-44.0	9.4
3274.2.1.10F	Bush Hill	Bed 1A	Interior	1992	4.3	-2.3	-40.1	9.5
3274.2.1.11F	Bush Hill	Bed 1A	Interior	1992	1.1	-1.2	-42.9	8.9
3274.2.1.13F	Bush Hill	Bed 1A	Interior	1992	2.1	-1.6	-47.0	7.8
3274.3.1(2).03F	Bush Hill	Bed 1A	Interior	1992	4.5	-2.5	-42.5	9.5
3274.3.1(2).05F	Bush Hill	Bed 1A	Interior	1992	2.7	-1.8	-41.0	9.0
3274.3.1(2).06F	Bush Hill	Bed 1A	Interior	1992	10.7	-4.1	-41.0	11.9
3274.3.1(2).08F	Bush Hill	Bed 1A	Interior	1992	7.6	-2.5	-41.8	8.5
3274.3.1(2).12F	Bush Hill	Bed 1A	Interior	1992	12.8	-3.3	-41.4	9.6
3274.3.1(2).15F	Bush Hill	Bed 1A	Interior	1992	6.1	-3.5	-42.1	11.3
3274.3.1.02F	Bush Hill	Bed 1A	Interior	1992	4.2	-1.5	-43.9	6.9
3274.3.1.17F	Bush Hill	Bed 1A	Interior	1992	35.9	-4.2	-40.7	9.9
3274.3.1.20F	Bush Hill	Bed 1A	Interior	1992	2.1	-1.4	-41.6	8.5
3274.1.1.01F	Bush Hill	Bed 1A	Edge	1992	11.0	-4.3	-39.9	12.7
3274.1.1.04F	Bush Hill	Bed 1A	Edge	1992	3.5	-2.0	-41.3	9.0
3274.1.1.06F	Bush Hill	Bed 1A	Edge	1992	2.8	-1.5	-41.5	8.2
3274.1.1.08F	Bush Hill	Bed 1A	Edge	1992	2.2	-1.4	-39.7	8.9
3274.1.1.11F	Bush Hill	Bed 1A	Edge	1992	11.9	-2.6	-39.6	8.5
3269.1.1.02F	Bush Hill	Bed 1	Edge	1992	26.3	-3.7	-38.3	10.0
3269.1.1.06F	Bush Hill	Bed 1	Edge	1992	5.3	-2.4	-39.3	9.7
3269.1.1.09F	Bush Hill	Bed 1	Edge	1992	21.0	-5.7	-40.1	14.8
3269.1.1.10F	Bush Hill	Bed 1	Edge	1992	9.7	-4.0	-40.9	11.8
3269.2.1(2).03F	Bush Hill	Bed 1	Edge	1992	18.7	-5.3	-36.7	15.3

A.5 Brine Pool 1989 North Soft Tissue

Table A.5: All soft tissue specimens for Brine Pool 1989 North sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	δ13C (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
2598.10.1(3).05F	Brine Pool	North	Interior	1989	-13.0	-58.8	9.2	570	2454	71	11	48	1.4
2598.10.1.01F	Brine Pool	North	Interior	1989	-13.4	-61.8	9.9	584	2501	69	11	47	1.3
2598.10.1.02f	Brine Pool	North	Interior	1989	-12.9	-59.0	9.0	715	3011	84	12	49	1.4
2598.10.1.03f	Brine Pool	North	Interior	1989	-14.7	-61.3	13.8	562	2493	88	10	45	1.6
2598.10.1.04f	Brine Pool	North	Interior	1989	-15.6	-65.1	9.2	802	3348	97	12	48	1.4
2598.9.1(2).01F	Brine Pool	North	Interior	1989	-17.3	-74.6	11.0	717	2727	76	14	52	1.4
2598.9.1(2).02F	Brine Pool	North	Interior	1989	-16.4	-69.2	11.1	627	2798	70	12	53	1.3
2598.9.1(2).03F	Brine Pool	North	Interior	1989	-16.9	-69.0	12.0	935	3595	96	14	52	1.4
2598.9.1(2).04F	Brine Pool	North	Interior	1989	-16.8	-71.6	12.1	828	3158	86	13	50	1.3
2598.9.1(2).05F	Brine Pool	North	Interior	1989	-16.9	-70.7	11.2	826	3201	86	13	50	1.3
2598.9.1(2).06F	Brine Pool	North	Interior	1989	-17.8	-77.8	10.2	795	3050	82	13	50	1.3
2598.9.1(2).07F	Brine Pool	North	Interior	1989	-17.8	-77.3	11.0	665	2561	70	13	50	1.4
2598.9.1(2).08F	Brine Pool	North	Interior	1989	-17.3	-73.8	11.8	797	3036	83	13	49	1.3
2598.9.1(2).09F	Brine Pool	North	Interior	1989	-17.0	-76.1	11.3	687	2626	70	13	48	1.3
2598.9.1(2).10F	Brine Pool	North	Interior	1989	-17.5	-77.1	11.2	786	2987	82	13	49	1.3
2598.9.1.01F	Brine Pool	North	Interior	1989	-17.7	-71.4	11.5	856	3164	87	13	49	1.3
2598.9.1.02F	Brine Pool	North	Interior	1989	-17.3	-73.1	11.1	712	2785	73	13	50	1.3
2598.9.1.03F	Brine Pool	North	Interior	1989	-17.3	-80.9	10.5	714	2739	74	13	49	1.3
2598.9.1.04f	Brine Pool	North	Interior	1989	-16.5	-69.9	12.1	797	3012	82	13	49	1.3
2598.9.1.05f	Brine Pool	North	Interior	1989	-16.2	-67.0	12.3	763	2920	80	13	50	1.4
2598.11.1(2).01F	Brine Pool	North	Edge	1989	-13.0	-59.5	8.2	721	2862	83	12	48	1.4
2598.11.1(2).02F	Brine Pool	North	Edge	1989	-13.4	-60.4	7.2	542	2296	63	11	46	1.3
2598.11.1(2).03F	Brine Pool	North	Edge	1989	-13.8	-59.1	6.3	626	2528	71	12	50	1.4
2598.11.1.01F	Brine Pool	North	Edge	1989	-13.5	-59.2	6.9	730	3028	85	12	50	1.4
2598.11.1.04F	Brine Pool	North	Edge	1989	-13.6	-59.8	8.0	718	2902	82	12	49	1.4
2598.11.1.05F	Brine Pool	North	Edge	1989	-15.9	-61.3	7.1	727	2843	83	12	48	1.4
2598.11.1.06F	Brine Pool	North	Edge	1989	-16.4	-63.3	6.5	813	3126	88	13	48	1.4
2598.12.1(2).01F	Brine Pool	North	Edge	1989	-13.8	-57.7	5.6	638	2424	76	12	47	1.5
2598.12.1(2).02F	Brine Pool	North	Edge	1989	-14.9	-61.1		628	2439		13	49	
2598.12.1(2).03F	Brine Pool	North	Edge	1989	-15.3	-61.3	4.7	741	2810	83	13	49	1.4
2598.12.1.01F	Brine Pool	North	Edge	1989	-14.0	-60.0	4.7	746	3132	100	11	46	1.5
2598.12.1.02F	Brine Pool	North	Edge	1989	-13.5	-58.4	5.3	713	2759	94	11	44	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.1	-57.4	5.7	597	2377	79	12	48	1.6
2598.12.1.03F	Brine Pool	North	Edge	1989	-11.9	-57.5	6.1	696	2790	92	12	47	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.0	-57.5	5.8	758	3065	98	12	48	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-11.9	-57.5	6.1	696	2790	92	12	47	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.0	-57.5	5.8	758	3065	98	12	48	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-11.9	-57.5	4.6	671	2664	86	12	47	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.0	-57.5	5.0	795	3127	103	12	47	1.5
2598.12.1.03F	Brine Pool	North	Edge	1989	-11.8	-57.4	5.8	781	3103	93	12	47	1.4
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.1	-56.1	6.1	783	3190	99	11	46	1.4
2598.12.1.03F	Brine Pool	North	Edge	1989	-12.0	-57.4	5.9	822	3305	107	12	48	1.5

A.6 Brine Pool 1989 South Soft Tissue

Table A.6:All soft tissue specimens for the Brine Pool 1989 South sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
2598.2.1.11F	Brine Pool	South	Interior	1989	-14.1	-57.0	2.7	574	2097	74	10	38	1.3
2598.4.1.01F	Brine Pool	South	Interior	1989	-14.6	-59.0	8.6	711	2743	76	13	50	1.4
2598.4.1.02F	Brine Pool	South	Interior	1989	-14.9	-60.8	12.0	638	2484	72	12	47	1.4
2598.4.1.03F	Brine Pool	South	Interior	1989	-14.8	-60.4	11.2	717	2738	83	12	47	1.4
2598.4.1.04f	Brine Pool	South	Interior	1989	-15.0	-61.9	10.3	843	3143	94	13	48	1.4
2598.4.1.05f	Brine Pool	South	Interior	1989	-15.9	-62.3	10.7	763	2828	85	13	47	1.4
2598.5.1(2).01F	Brine Pool	South	Interior	1989	-15.5	-62.0	12.0	727	2792	78	13	49	1.4
2598.5.1(2).02F	Brine Pool	South	Interior	1989	-15.1	-59.1	12.0	882	3379	90	13	49	1.3
2598.5.1(2).03F	Brine Pool	South	Interior	1989	-14.0	-61.7	12.3	822	3214	82	13	50	1.3
2598.5.1(2).04F	Brine Pool	South	Interior	1989	-15.7	-62.1	12.3	707	2808	70	12	50	1.2
2598.5.1(2).05F	Brine Pool	South	Interior	1989	-16.4	-63.1	12.0	655	2476	67	13	48	1.3
2598.5.1.01F	Brine Pool	South	Interior	1989	-15.4	-62.2	12.5	730	2910	76	12	47	1.2
2598.5.1.02f	Brine Pool	South	Interior	1989	-15.1	-60.8	13.1	659	2713	76	11	47	1.3
2598.7.1(2).01F	Brine Pool	South	Edge	1989	-14.7	-62.1	12.9	851	3403	91	12	50	1.3
2598.7.1(2).02F	Brine Pool	South	Edge	1989	-14.8	-60.6	13.6	644	2834	77	11	47	1.3
2598.7.1(2).03F	Brine Pool	South	Edge	1989	-15.5	-62.1	13.8	601	2618	69	11	49	1.3
2598.7.1.01F	Brine Pool	South	Edge	1989	-15.3	-64.6	13.3	694	2798	78	12	48	1.3
2598.8.1.07f	Brine Pool	South	Edge	1989	-16.4	-67.2	13.7	731	2732	76	13	50	1.4
2598.8.1.8F	Brine Pool	South	Edge	1989	-16.0	-67.1	12.7	716	2616	74	13	48	1.4

A.7 Brine Pool 1991 Soft Tissue

Table A.7: All soft tissue specimens from the Brine Pool 1991 sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3145.3.1.01F	Brine Pool	South	Interior	1991	-13.5	-58.6	11.2	830	3114	86	14	52	1.4
3145.3.1.02F	Brine Pool	South	Interior	1991	-14.7	-61.1	11.7	640	2663	66	12	51	1.3
3145.3.1.03F	Brine Pool	South	Interior	1991	-13.7	-59.6	10.7	915	3546	96	13	51	1.4
3145.3.1.04F	Brine Pool	South	Interior	1991	-14.5	-61.9	11.1	880	3356	89	14	53	1.4
3145.3.1.05F	Brine Pool	South	Interior	1991	-16.0	-65.0	11.0	619	2804	64	11	51	1.2
3145.3.1.06F	Brine Pool	South	Interior	1991	-16.5	-65.2	10.6	878	3281	87	14	51	1.3
3145.3.1.07F	Brine Pool	South	Interior	1991	-16.0	-64.3	10.8	761	3140	76	13	52	1.3
3145.3.1.08F	Brine Pool	South	Interior	1991	-16.0	-63.6	10.9	790	2952	73	14	53	1.3
3145.5.1.01F	Brine Pool	North	Edge	1991	3.8	-57.7	-0.9	867	3091	77	13	48	1.2
3145.5.1.02F	Brine Pool	North	Edge	1991	4.4	-56.7	1.8	696	2476	61	14	49	1.2
3145.5.1.03F	Brine Pool	North	Edge	1991	4.4	-59.3	1.9	861	3016	74	14	48	1.2
3145.5.1.04F	Brine Pool	North	Edge	1991	3.3	-57.7	1.6	764	2798	66	13	48	1.1
3145.5.1.05F	Brine Pool	North	Edge	1991	3.8	-56.8	-1.1	656	2460	60	13	48	1.2
3145.6.1.1F	Brine Pool	North	Interior	1991	-12.1	-51.3	10.3	795	3406	63	14	60	1.1
3145.6.1.02F	Brine Pool	North	Interior	1991	-15.6	-62.1	11.3	791	3083	83	12	47	1.3
3145.6.1.03F	Brine Pool	North	Interior	1991	-15.7	-60.9	12.0	641	2355	66	12	46	1.3
3145.6.1.04F	Brine Pool	North	Interior	1991	-15.5	-61.2	12.0	660	2436	68	12	46	1.3
3145.6.1.05F	Brine Pool	North	Interior	1991	-15.3	-60.9	11.8	760	2938	78	12	47	1.3
3145.6.1.06F	Brine Pool	North	Interior	1991	-15.1	-59.4	11.8	805	2961	82	13	48	1.3
3145.6.1.07F	Brine Pool	North	Interior	1991	-14.8	-60.3	12.7	597	2482	68	11	47	1.3
3145.6.1.08F	Brine Pool	North	Interior	1991	-14.4	-60.7	11.9	728	3212	71	11	47	1.0
3145.6.1.09F	Brine Pool	North	Interior	1991	-14.8	-59.5	12.6	843	3064	77	13	48	1.2
3145.6.1.10F	Brine Pool	North	Interior	1991	-14.5	-59.6	11.9	899	3184	81	14	49	1.2
3145.6.1.11F	Brine Pool	North	Interior	1991	-14.0	-61.3	11.6	696	2693	67	12	47	1.2
3145.7.1.01F	Brine Pool	North	Interior	1991	-14.8	-62.4	11.7	606	2736	63	10	47	1.1
3145.7.1.02F	Brine Pool	North	Interior	1991	-14.6	-61.3	12.0	733	3007	74	12	47	1.2
3145.7.1.03F	Brine Pool	North	Interior	1991	-14.9	-61.5	12.8	831	3122	87	13	48	1.4

A.8 Brine Pool 2006 Soft Tissue

Table A.8: All soft tissue specimens for the Brine Pool 2006 Sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3522.02F	Brine Pool	South		2006	-16.9	-64.1	11.1	958	3516	114	14	51	1.7
3522.03F	Brine Pool	South		2006	-16.8	-68.6	12.2	459	1640	58	13	46	1.6
3522.05F	Brine Pool	South		2006	-16.8	-65.9	9.0	919	3317	106	14	52	1.7
3522.08f	Brine Pool	South		2006	-16.4	-65.2	11.6	894	3280	103	14	53	1.7
3522.09f	Brine Pool	South		2006	-16.5	-62.7	12.0	769	2789	90	15	53	1.7
3522.11f	Brine Pool	South		2006	-15.1	-63.2	11.7	755	2755	89	14	51	1.7
3522.12f	Brine Pool	South		2006	-16.3	-64.6	11.1	775	2801	88	14	50	1.6
3522.13F	Brine Pool	South		2006	-16.9	-66.0	9.6	722	2581	81	14	50	1.6
3522.18F	Brine Pool	South		2006	-16.3	-61.1	11.3	757	2792	92	13	48	1.6
3522.19f	Brine Pool	South		2006	-16.5	-63.1	12.3	786	2815	92	14	50	1.6
3522.20F	Brine Pool	South		2006	-17.3	-66.5	11.4	782	2850	91	15	53	1.7
3522.21F	Brine Pool	South		2006	-16.8	-69.2	10.2	656	2341	72	13	46	1.4
3522.23f	Brine Pool	South		2006	-16.7	-61.6	11.7	717	2531	81	14	48	1.6
3522.25F	Brine Pool	South		2006	-17.2	-65.1	11.6	648	2447	75	12	45	1.4
3522.30f	Brine Pool	South		2006	-16.5	-67.3	11.8	735	2651	87	14	49	1.6
3522.34F	Brine Pool	South		2006	-16.2	-62.3	11.6	840	3034	97	13	46	1.5
3522.35f	Brine Pool	South		2006	-15.5	-57.5	9.5	708	2596	84	13	48	1.6
3522.36F	Brine Pool	South		2006	-16.8	-64.3	11.9	736	2819	88	12	47	1.5
3522.39F	Brine Pool	South		2006	-17.6	-69.4	11.9	658	2427	75	12	46	1.4
3522.45F	Brine Pool	South		2006	-16.7	-68.1	11.8	638	2363	73	12	46	1.4
3522.46F	Brine Pool	South		2006	-18.0	-69.3	7.3	711	2623	96	11	42	1.5
3522.47F	Brine Pool	South		2006	-15.7	-61.6	10.6	774	2972	104	11	43	1.5
3522.48F	Brine Pool	South		2006	-15.2	-60.2	10.9	885	3314	99	13	49	1.5
3522.51f	Brine Pool	South		2006	-16.2	-66.4	11.8	971	3571	113	14	51	1.6
3522.52F	Brine Pool	South		2006	-15.5	-59.0	10.4	612	2262	70	10	37	1.1
3522.54f	Brine Pool	South		2006	-16.2	-63.3	12.6	822	2930	91	15	53	1.6
3522.55F	Brine Pool	South		2006	-16.7	-63.9	10.6	660	2462	79	12	46	1.5
3522.60F	Brine Pool	South		2006	-15.6	-62.7	10.5	892	3371	105	13	48	1.5
3522.61F	Brine Pool	South		2006	-17.3	-67.0	11.2	899	3297	101	13	48	1.5
3522.64F	Brine Pool	South		2006	-16.3	-68.0	11.4	805	3110	98	12	48	1.5
3522.65F	Brine Pool	South		2006	-15.9	-61.8	9.5	792	3057	93	13	49	1.5
3522.70F	Brine Pool	South		2006	-16.6	-65.4	10.9	656	2444	78	13	48	1.5
3522.71F	Brine Pool	South		2006	-17.3	-70.7	7.2	797	2964	98	13	47	1.5
3522.76F	Brine Pool	South		2006	-16.4	-62.2	11.4	723	2683	82	13	49	1.5
3522.77F	Brine Pool	South		2006	-16.8	-70.7	8.5	771	2896	99	12	44	1.5
3521.02f	Brine Pool	North		2006	-15.1	-56.8	10.1	717	2717	84	14	51	1.6
3521.03f	Brine Pool	North		2006	-15.1	-56.2	10.4	820	2965	108	13	47	1.7
3521.04f	Brine Pool	North		2006	-15.0	-56.9	10.9	841	3191	118	12	46	1.7
3521.05f	Brine Pool	North		2006	-15.1	-57.6	11.3	833	3100	134	12	45	1.9
3521.06f	Brine Pool	North		2006	-15.3	-56.8	10.5	936	3421	107	14	50	1.6
3521.07f	Brine Pool	North		2006	-14.7	-57.9	10.8	836	3102	109	13	47	1.6
3521.08f	Brine Pool	North		2006	-14.8	-58.0	11.8	836	3158	102	13	50	1.6
3521.09f	Brine Pool	North		2006	-15.4	-57.5	9.1	929	3316	116	14	49	1.7
3521.10F	Brine Pool	North		2006	-12.9	-54.3	10.7	713	2662	57	11	42	0.9
3521.13F	Brine Pool	North		2006	-13.5	-55.2	10.5	688	2784	61	13	55	1.2
3521.14F	Brine Pool	North		2006	-15.0	-57.0	11.8	706	2403	69	11	36	1.0

A.9 Bush Hill 1991 Soft Tissue

Table A.9: All soft tissue specimens for the Bush Hill 1991Bed 1 sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3139.1.1.01F	Bush Hill	Bed 1	Edge	1991	6.4	-35.7	-5.0	841	2989	87	14	50	1.4
3139.1.1.02F	Bush Hill	Bed 1	Edge	1991	4.6	-36.7	0.0	813	2919	95	14	49	1.6
3139.1.1.03F	Bush Hill	Bed 1	Edge	1991	5.2	-35.5	-5.1	982	3404	108	14	49	1.5
3139.1.1.04F	Bush Hill	Bed 1	Edge	1991	6.1	-35.7	-3.0	932	3302	107	14	49	1.6
3139.1.1.05F	Bush Hill	Bed 1	Edge	1991	3.7	-36.8	-1.8	730	2592	75	13	47	1.4
3139.1.1.06F	Bush Hill	Bed 1	Edge	1991	5.2	-35.7	-2.6	742	2684	74	13	46	1.3
3139.1.1.07F	Bush Hill	Bed 1	Edge	1991	5.4	-35.7	-4.2	840	2966	84	13	47	1.3
3139.1.1.08F	Bush Hill	Bed 1	Edge	1991	4.4	-36.5	-2.6	770	2808	78	13	47	1.3
3139.1.1.09F	Bush Hill	Bed 1	Edge	1991	4.9	-35.6	-1.5	802	2954	79	13	49	1.3
3139.1.1.10F	Bush Hill	Bed 1	Edge	1991	4.8	-36.2	-5.9	731	2612	73	14	49	1.4
3139.1.1.11F	Bush Hill	Bed 1	Edge	1991	5.0	-36.1	-2.0	874	3114	93	13	47	1.4
3139.1.1.12F	Bush Hill	Bed 1	Edge	1991	5.1	-36.4	-3.5	760	2754	78	13	48	1.4
3139.1.1.13F	Bush Hill	Bed 1	Edge	1991	4.8	-36.6	-3.3	914	3290	93	13	48	1.4
3139.1.1.14F	Bush Hill	Bed 1	Edge	1991	4.3	-36.2	-4.1	908	3194	89	14	49	1.4
3139.1.1.15F	Bush Hill	Bed 1	Edge	1991	6.1	-36.6	-1.1	767	2800	79	14	49	1.4
3139.2.1.01F	Bush Hill	Bed 1	Interior	1991	6.4	-35.8	-6.6	759	2669	80	12	43	1.3
3139.2.1.02F	Bush Hill	Bed 1	Interior	1991	5.3	-35.8	-1.7	922	3139	86	15	50	1.4
3139.2.1.03F	Bush Hill	Bed 1	Interior	1991	5.2	-36.1	0.3	869	3110	98	16	57	1.8
3139.2.1.04F	Bush Hill	Bed 1	Interior	1991	5.5	-35.7	-1.9	837	3010	97	14	49	1.6
3139.2.1.05F	Bush Hill	Bed 1	Interior	1991	5.3	-35.8	-2.8	896	3075	89	14	50	1.4
3139.2.1.08F	Bush Hill	Bed 1	Interior	1991	5.7	-37.9	-0.1	796	2821	85	14	51	1.5
3139.2.1.09F	Bush Hill	Bed 1	Interior	1991	5.5	-35.8	-2.6	630	2235	68	14	48	1.5
3139.2.1.10F	Bush Hill	Bed 1	Interior	1991	5.1	-36.5	1.1	879	3224	90	13	49	1.4
3139.2.1.13F	Bush Hill	Bed 1	Interior	1991	4.9	-37.0	-1.3	747	2645	73	14	49	1.3
3139.2.1.14F	Bush Hill	Bed 1	Interior	1991	5.6	-36.8	0.1	792	2916	81	13	49	1.4
3139.3.1.03f	Bush Hill	Bed 1	Interior	1991	5.5	-36.0	-3.7	884	3188	90	14	51	1.5
3139.3.1.05F	Bush Hill	Bed 1	Interior	1991	5.6	-36.1	-4.9	740	2777	75	13	50	1.4
3139.3.1.07F	Bush Hill	Bed 1	Interior	1991	5.6	-35.6	-2.1	714	2805	83	12	48	1.4
3139.3.1.08F	Bush Hill	Bed 1	Interior	1991	3.8	-36.3	-3.6	674	2451	70	13	48	1.4
3139.3.1.09F	Bush Hill	Bed 1	Interior	1991	5.2	-36.7	-0.2	706	2585	67	13	48	1.2
3139.3.1.12F	Bush Hill	Bed 1	Interior	1991	5.0	-35.9	-4.8	715	2615	65	13	47	1.2
3139.3.1.13F	Bush Hill	Bed 1	Interior	1991	5.4	-36.2	-3.6	824	3002	82	13	47	1.3
3139.3.1.15F	Bush Hill	Bed 1	Interior	1991	6.2	-36.2	-2.8	675	2506	69	13	47	1.3
3139.3.1.20F	Bush Hill	Bed 1	Interior	1991	5.4	-36.9	-1.3	842	3125	82	13	48	1.3
3139.3.1.21F	Bush Hill	Bed 1	Interior	1991	4.6	-36.0	-2.8	906	3268	79	13	47	1.1
3139.3.1.23F	Bush Hill	Bed 1	Interior	1991	6.1	-36.7	-1.5	825	3058	81	13	48	1.3

A.10 Bush Hill 1991 Bed 2 Soft Tissue

Table A.10: All soft tissue specimens for the Bush Hill 1991 Bed 2 sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3139.4.1.01F	Bush Hill	Bed 2	Interior	1991	5.4	-35.7	-3.5	672	2339	66	13	47	1.3
3139.4.1.02F	Bush Hill	Bed 2	Interior	1991	4.8	-36.3	-4.2	671	2396	64	13	46	1.2
3139.4.1.03F	Bush Hill	Bed 2	Interior	1991	4.0	-36.2	-4.4	738	2640	71	13	46	1.2
3139.4.1.04F	Bush Hill	Bed 2	Interior	1991	5.6	-36.3	-3.2	754	2712	76	13	48	1.3
3139.4.1.05F	Bush Hill	Bed 2	Interior	1991	3.6	-36.8	-1.6	867	3040	85	13	47	1.3
3139.4.1.06F	Bush Hill	Bed 2	Interior	1991	4.3	-36.7	-4.4	719	2519	70	13	47	1.3
3139.4.1.07F	Bush Hill	Bed 2	Interior	1991	5.7	-35.7	-3.5	730	2566	71	13	47	1.3
3139.4.1.08F	Bush Hill	Bed 2	Interior	1991	5.4	-36.1	-4.8	722	2592	79	13	47	1.4
3139.4.1.13F	Bush Hill	Bed 2	Interior	1991	4.8	-36.2	-3.2	755	2714	73	13	48	1.3
3139.4.1.15F	Bush Hill	Bed 2	Interior	1991	4.8	-35.9	-3.5	790	2791	74	13	46	1.2
3139.4.1.16F	Bush Hill	Bed 2	Interior	1991	5.7	-36.3	-1.0	739	2612	66	13	47	1.2
3139.4.1.17F	Bush Hill	Bed 2	Interior	1991	5.5	-35.9	-3.8	868	3108	80	13	47	1.2
3139.5.1.2F	Bush Hill	Bed 2	Interior	1991	5.0	-37.5	5.6	664	2349	55	10	36	0.8
3139.5.1.03F	Bush Hill	Bed 2	Interior	1991	6.2	-37.2	3.6	663	2460	60	12	46	1.1
3139.4.1.14F	Bush Hill	Bed 2	Interior	1991	0.4	-41.4	1.7	858	3583	66	16	68	1.3
3139.5.1.09F	Bush Hill	Bed 2	Interior	1991	5.4	-37.4	4.1	681	2468	60	13	46	1.1
3139.7.1.01F	Bush Hill	Bed 2	Edge	1991	3.2	-38.7	3.5	890	3197	93	13	47	1.4
3139.7.1.02F	Bush Hill	Bed 2	Edge	1991	3.0	-38.0	1.5	717	2617	78	13	47	1.4
3139.7.1.03F	Bush Hill	Bed 2	Edge	1991	3.3	-36.9	0.5	832	2990	90	13	46	1.4
3139.7.1.04F	Bush Hill	Bed 2	Edge	1991	4.0	-37.3	3.6	835	3103	80	12	45	1.2
3139.7.1.05F	Bush Hill	Bed 2	Edge	1991	5.2	-37.9	3.9	722	2628	81	13	46	1.4
3139.7.1.06F	Bush Hill	Bed 2	Edge	1991	4.1	-37.6	3.5	712	2661	74	12	46	1.3
3139.7.1.07F	Bush Hill	Bed 2	Edge	1991	4.1	-38.1	4.0	744	2690	74	13	48	1.3
3139.7.1.08F	Bush Hill	Bed 2	Edge	1991	3.0	-38.0	2.1	706	2518	67	13	47	1.3
3139.7.1.09F	Bush Hill	Bed 2	Edge	1991	2.6	-37.4	1.8	611	2235	64	12	45	1.3
3139.7.1.10F	Bush Hill	Bed 2	Edge	1991	4.9	-37.8	2.3	703	2559	72	13	46	1.3
3139.7.1.11F	Bush Hill	Bed 2	Edge	1991	4.4	-38.2	4.6	728	2672	72	13	48	1.3
3139.7.1.12F	Bush Hill	Bed 2	Edge	1991	4.4	-37.5	4.3	752	2750	72	13	47	1.2
3139.7.1.13F	Bush Hill	Bed 2	Edge	1991	4.6	-39.0	6.4	720	2700	81	13	47	1.4
3139.7.1.14F	Bush Hill	Bed 2	Edge	1991	3.8	-40.8	4.3	816	3016	80	13	48	1.3
3139.7.1.15F	Bush Hill	Bed 2	Edge	1991	4.4	-37.4	5.3	866	3122	86	13	48	1.3

A.11 Bush Hill 1992 Bed 1 Soft Tissue

Table A.11: All soft tissue specimens for the Bush Hill 1992 Bed 1 sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3269.1.1(2).01F	Bush Hill	Bed 1	Edge	1992	3.0	-39.4	1.4	685	2448	62	13	47	1.2
3269.1.1(2).02F	Bush Hill	Bed 1	Edge	1992	4.1	-38.7	2.0	701	2567	70	13	46	1.3
3269.1.1(2).05F	Bush Hill	Bed 1	Edge	1992	2.7	-39.0	-0.9	763	3012	75	12	47	1.2
3269.1.1(2).06F	Bush Hill	Bed 1	Edge	1992	4.7	-38.9	0.7	586	2504	55	11	48	1.1
3269.1.1(2).07F	Bush Hill	Bed 1	Edge	1992	4.1	-38.4	1.6	797	3133	79	12	47	1.2
3269.1.1(2).08F	Bush Hill	Bed 1	Edge	1992	4.5	-39.7	2.2	754	2754	73	13	46	1.2
3269.2.1(2).10F	Bush Hill	Bed 1	Edge	1992	3.7	-39.6	-1.2	574	2328	54	11	46	1.1
3269.2.1(2).12F	Bush Hill	Bed 1	Edge	1992	4.2	-39.0	0.8	621	2787	60	10	45	1.0
3269.1.1.01F	Bush Hill	Bed 1	Edge	1992	5.5	-37.9	0.0	701	2555	72	12	42	1.2
3269.1.1.02F	Bush Hill	Bed 1	Edge	1992	4.8	-38.3	-0.4	783	2855	80	13	46	1.3
3269.1.1.04F	Bush Hill	Bed 1	Edge	1992	5.2	-38.0	1.2	688	2545	69	12	45	1.2
3269.1.1.06F	Bush Hill	Bed 1	Edge	1992	3.1	-39.3	-0.1	785	2800	77	13	46	1.3
3269.1.1.07F	Bush Hill	Bed 1	Edge	1992	5.5	-38.0	0.3	855	3097	87	13	48	1.3
3269.1.1.08F	Bush Hill	Bed 1	Edge	1992	4.1	-38.0	-0.2	777	2783	74	13	46	1.2
3269.1.1.09F	Bush Hill	Bed 1	Edge	1992	4.0	-40.1	-1.0	798	2889	78	12	45	1.2
3269.1.1.10F	Bush Hill	Bed 1	Edge	1992	3.3	-40.9	-0.1	759	2819	70	13	47	1.2
3269.2.1(2).03F	Bush Hill	Bed 1	Edge	1992	4.9	-36.7	0.8	747	3143	81	11	48	1.2
3269.2.1(2).04F	Bush Hill	Bed 1	Edge	1992	4.1	-39.2	0.5	630	2547	65	11	46	1.2
3269.2.1(2).05F	Bush Hill	Bed 1	Edge	1992	4.1	-38.7	0.3	668	2535	67	13	48	1.3
3269.2.1(2).06F	Bush Hill	Bed 1	Edge	1992	3.5	-37.9	1.2	847	3211	88	13	48	1.3
3269.2.1(2).07F	Bush Hill	Bed 1	Edge	1992	3.9	-39.6	-0.1	735	3141	77	11	48	1.2
3269.2.1(2).08F	Bush Hill	Bed 1	Edge	1992	4.0	-37.9	0.9	631	2548	63	12	47	1.2
3269.2.1(2).09F	Bush Hill	Bed 1	Edge	1992	4.0	-39.2	0.5	652	3014	65	10	46	1.0
3269.2.1(2).11F	Bush Hill	Bed 1	Edge	1992	3.7	-39.0	0.4	564	2560	56	10	45	1.0
3269.11.1.01F	Bush Hill	Bed 1	Interior	1992	4.9	-38.3	0.8	826	2989	80	13	48	1.3
3269.11.1.02F	Bush Hill	Bed 1	Interior	1992	5.4	-37.1	0.4	829	2953	80	13	48	1.3
3269.11.1.03F	Bush Hill	Bed 1	Interior	1992	4.0	-39.1	0.8	850	3045	84	13	47	1.3
3269.11.1.04F	Bush Hill	Bed 1	Interior	1992	3.8	-39.0	0.1	898	3211	84	13	48	1.2
3269.11.1.05F	Bush Hill	Bed 1	Interior	1992	5.0	-38.6	0.6	895	3138	78	14	48	1.2
3269.11.1.06F	Bush Hill	Bed 1	Interior	1992	3.9	-38.9	-0.1	708	2472	61	14	47	1.2
3269.11.1.07F	Bush Hill	Bed 1	Interior	1992	5.2	-38.5	-0.5	813	2865	70	13	47	1.1
3269.11.1.08F	Bush Hill	Bed 1	Interior	1992	5.1	-38.4	1.6	791	2883	75	13	48	1.3
3269.12.1.01F	Bush Hill	Bed 1	Interior	1992	5.3	-37.1	0.8	725	2540	67	14	48	1.3
3269.12.1.02F	Bush Hill	Bed 1	Interior	1992	3.4	-37.9	0.5	741	2571	69	14	48	1.3
3269.12.1.3F	Bush Hill	Bed 1	Interior	1992	3.3	-40.7	2.7	618	1937	51	12	37	1.0

A.12 Bush Hill 1992 Bed 1A Soft Tissue

Table A.12: All soft tissue specimens for the Bush Hill 1992 Bed 1A sampling.

Sample ID	Site	Bed	Position	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	N (mg)	C (mg)	S (mg)	%N	%C	%S
3274.1.1.01F	Bush Hill	Bed 1A	Edge	1992	3.7	-39.9	7.2	826	3251	78	13	52	1.2
3274.1.1.04F	Bush Hill	Bed 1A	Edge	1992	2.1	-41.3	7.0	718	2857	67	12	46	1.1
3274.1.1.06F	Bush Hill	Bed 1A	Edge	1992	3.0	-41.5	6.7	819	3067	76	12	46	1.2
3274.1.1.08F	Bush Hill	Bed 1A	Edge	1992	3.4	-39.7	6.5	734	2644	68	13	47	1.2
3274.1.1.10F	Bush Hill	Bed 1A	Edge	1992	5.5	-37.3	6.4	817	3589	88	12	53	1.3
3274.1.1.11F	Bush Hill	Bed 1A	Edge	1992	2.7	-39.6	9.0	613	2346	56	12	46	1.1
3274.2.1.02F	Bush Hill	Bed 1A	Interior	1992	2.1	-43.5	7.0	647	2318	60	13	46	1.2
3274.2.1.04F	Bush Hill	Bed 1A	Interior	1992	1.8	-43.5	7.4	885	3175	82	13	46	1.2
3274.2.1.09F	Bush Hill	Bed 1A	Interior	1992	2.4	-44.0	7.1	725	2604	65	13	48	1.2
3274.2.1.10F	Bush Hill	Bed 1A	Interior	1992	3.2	-40.1	7.7	916	3253	85	13	47	1.2
3274.2.1.11F	Bush Hill	Bed 1A	Interior	1992	1.5	-42.9	6.9	862	3028	82	13	46	1.2
3274.2.1.13F	Bush Hill	Bed 1A	Interior	1992	1.6	-47.0	6.3	791	2784	72	13	46	1.2
3274.3.1(2).03F	Bush Hill	Bed 1A	Interior	1992	2.2	-42.5	5.9	643	2584	67	12	47	1.2
3274.3.1(2).05F	Bush Hill	Bed 1A	Interior	1992	2.5	-41.0	7.0	666	2409	63	13	47	1.2
3274.3.1(2).06F	Bush Hill	Bed 1A	Interior	1992	4.4	-41.0	6.3	788	3011	79	12	48	1.3
3274.3.1(2).08F	Bush Hill	Bed 1A	Interior	1992	3.7	-41.8	7.2	680	2557	66	13	47	1.2
3274.3.1(2).12F	Bush Hill	Bed 1A	Interior	1992	3.4	-41.4	7.0	710	2733	73	12	46	1.2
3274.3.1(2).15F	Bush Hill	Bed 1A	Interior	1992	3.2	-42.1	6.4	670	2564	68	12	46	1.2
3274.3.1.02F	Bush Hill	Bed 1A	Interior	1992	2.2	-43.9	4.3	861	3143	94	13	46	1.4
3274.3.1.17F	Bush Hill	Bed 1A	Interior	1992	4.4	-40.7	8.4	693	2653	66	13	48	1.2
3274.3.1.20F	Bush Hill	Bed 1A	Interior	1992	2.1	-41.6	6.6	845	3045	92	13	46	1.4

4 Vita

Philip Martin Riekenberg was born in Leander, Texas, the son of Martin Jay Riekenberg and Martha Ann Krischke. He completed a Bachelor of Science in Biology with a concentration in freshwater and marine sciences at the University of Texas at Austin in 2006. Subsequently, he worked as a research associate for Brian Fry working with stable isotopes in estuarine settings for Louisiana State University for 5 years and was accepted to the graduate program at Louisiana State University as a graduate student in 2008.